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**THE EFFECT OF ENVIRONMENTAL VARIABLES
ON AMPHIBIAN BREEDING PHENOLOGY**

A thesis submitted in accordance with the requirements of the Open
University for the degree of

Doctor of Philosophy

In the discipline of

Life Sciences

by

Rachel Anne Grant (BSc. Hons, PG Dip)

Submitted on 31/10/11

Supervisors:

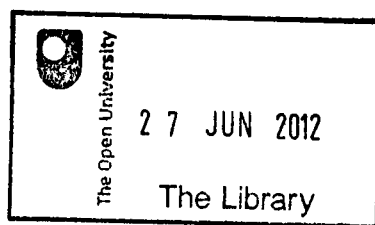
Prof. Tim Halliday, Dr Franco Andreone and Dr Mandy Dyson

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Abstract

Amphibian breeding phenology has generally been associated with temperature and rainfall, but these variables are not able to explain all of the variation in the timing of amphibian migrations, mating and spawning. This thesis examines some additional, previously under-acknowledged geophysical variables that may affect amphibian breeding phenology: lunar phase and the K-index of geomagnetic activity. A serendipitous observation of a large earthquake during the amphibian breeding season enabled a rare record of animal behaviour prior to an earthquake and led to an investigation into the effect of seismicity on amphibians. Data were collected on breeding migrations at three sites in the UK and Italy for frogs (*Rana temporaria*) and toads (*Bufo bufo*). Additional data were collated from published literature. Data on the arrivals of two newt species (*Triturus cristatus* and *Lissotriton helveticus*) were also analysed. Lunar phase was found to be important in *Rana temporaria* and *Bufo bufo*, with more individuals migrating, in amplexus and spawning around the full moon. Newts' response to the full moon was less clear. A meta-analysis of published data revealed that the effect of the lunar cycle on amphibians may be more prevalent than previously supposed and is species-specific,

depending on the unique ecology of each species. The effects of the K-index on amphibian reproduction are unclear because of the low number of days when geomagnetism was high. Five days before a large earthquake in L'Aquila, Italy the majority of toads left the breeding site, only re-appearing when the earthquake was over. Numbers of toads were significantly correlated with days since the earthquake but not with weather variables. Finally I attempted to use the variables of interest (two measures of moon phase, plus the K-index of magnetic activity), along with weather variables to construct statistical models of amphibian breeding phenology and to predict arrivals and spawning / amplexus in single years based on the models. This met with variable success; there was a high variability between years in the ability of the models to predict breeding phenology, which could be due to site-specific factors, unmeasured environmental variables, or an endogenous component to breeding phenology.



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Chapter 1: Introduction and literature review

1. 1 Aim of the study

The aim of this research is to consider the effects of previously under-represented geophysical variables that may affect amphibian reproduction, with particular emphasis on lunar phases, but also considering geomagnetism and seismic activity.

1.2 Background and literature review

1.2.1 Amphibians and their reproductive ecology

Amphibians are quadrupedal, generally small vertebrates and include salamanders, newts, frogs, toads and the limbless caecilians. They are ectothermic animals with at least one of their life history stages usually dependent on water (Prado et al. 2005). Amphibians moved from water to land in the Devonian period (350Ma) and underwent large adaptive radiation into numerous different groups with differing ecology and morphology. The current number of described amphibian species is 6,891,

a figure that is increasing by 1 or 2 species each week (Amphibiaweb; www.amphibiaweb.org, accessed Oct 25, 2011).

There is considerable diversity of reproductive strategies in amphibians (Duellman 1985, Hödl 1990). Reproductive strategies are aspects of a species' biology that ensure optimal numbers of offspring, including behaviour, morphology and physiology and these strategies are acted upon by natural selection, meaning that the final strategy may be a compromise between different selection pressures (Semlitsch 1985). Reproductive strategies typically consist of various elements including endogenous and exogenous control of gametogenesis, the number of females breeding, how often they ovulate, the duration of larval development, parental care and the size of clutches and eggs (Semlitsch 1985), all of which may also be affected by environmental constraints such as minimum temperatures.

Anurans (frogs and toads) in tropical and equatorial areas with high rainfall may reproduce all year round, producing several clutches of eggs, whereas those in temperate zones are constrained by low winter temperatures and summer drought and generally reproduce once a year, in spring. Much work has been done on temperate anurans of the genus *Bufo* and *Rana*, which

gather to breed for a short period each spring, and lay eggs which develop into aquatic tadpoles (Beebee and Griffiths 2000).

In anurans, breeding can be divided into two broad reproductive patterns. Those species with short breeding seasons, often called explosive breeders (Wells 1977, 2007) are characterised by females arriving at the breeding site synchronously, either at the same time or shortly after males, who actively compete for females in highly male-biased breeding assemblages. In contrast, prolonged breeders are characterised by males calling to attract females, often defending territories (Wells 1977, 2007). Explosive and prolonged breeding represent two extremes along a continuum of mating strategies.

Urodeles (salamanders and newts) normally reproduce once a year and generally have long breeding seasons. For example, in the UK the breeding migrations of *Lissotriton* and *Triturus* newts occur over a protracted period between January and April, after spending the winter in terrestrial refugia (Beebee and Griffiths 2000). If weather conditions are favourable newt arrivals will not be particularly synchronised, but after a prolonged cold period a mass migration can result (Beebee and Griffiths 2000). In some

species, there is also an autumn migration (Bell 1977; Verrell and Halliday 1985a). At the breeding sites male newts often outnumber females, resulting in competition for females, who arrive later and do not stay for as long at the breeding site, but the sex ratio sometimes approaches 1:1 (Verrell and Halliday 1985a). Newts have complex courtship rituals, which involve male display and release of pheromones, after which the male deposits one or more spermatophores that the female picks up (Halliday 1974).

In anurans, the testes have a simple structure, getting larger and heavier during spermatogenesis, with sperm cells maturing uniformly in temperate anurans, and less uniformly in tropical species that breed all year round (Roosen-Runge 1977). Oogenesis occurs when the primary oocytes divide by meiosis to form secondary oocytes and then polar bodies, accompanied by a ten-fold size increase (Lofts 1974). The oocytes then undergo vitellogenesis, whereby the nutrients needed for the developing embryo are gathered. In amphibians with distinct breeding seasons, Lofts (1974) identified four stages of oocytes at the beginning of the breeding season: 1. Cell nests that will form future follicles; 2. Previtellogenic follicles; 3. Follicles that are rapidly growing and undergoing vitellogenesis; 4.

Postvitellogenic mature ova. In most temperate amphibian species, oogenesis in females is completed before they enter hibernation, ready for the next breeding season (Wells 2007). Female toads (*Bufo bufo*) do not breed every year, and after oogenesis is completed they undergo a resting period for one to two years (Jørgensen 1984).

In many tropical or subtropical amphibians, spermatic and oogenic cycles are continuous, but in temperate regions low temperatures in winter impair the secretion of gonadotrophins by the pituitary gland, which inhibits this cycle (Paniagua et al. 1990). Spermatogenic and oogenic development are not synchronous with each other and may respond differently to environmental cues but as long as a certain temperature threshold has been reached many amphibians respond to rainfall, which appears to stimulate breeding in diverse species (Beebee and Griffiths 2000). Females' nutritional status can affect the number of eggs laid as well as their size (Jørgensen 1982; Tejedo 1992; Reading & Clarke 1995).

1.2.2 *Environmental control of reproduction in ectotherms*

Much of the work on the environmental control of reproduction in ectothermic animals has been done on fish (Bradford and Taylor 1987). It is useful to consider the hierarchy of variables to which fish respond, to gain insights into environmental control of reproduction in amphibians where less work has been done. At high latitudes, photoperiod and water temperature change distinctly with the seasons and these variables are important in controlling fish reproduction from the temperate to the polar zones (Takemura et al. 2004; Bradford and Taylor 1987). These regular fluctuations trigger initiation and synchronisation of various reproductive events in fishes (Pankhurst and Porter 2003). At high latitudes, seasonal reproduction occurs at the time of year that will be most beneficial for offspring survival (Bromage et al. 2001). The reproductive biology of the adults can also confine reproductive timing (Robertson 1991).

Sumpter (1990) observes that, in order that an environmental cue can control reproduction, it must have three properties:

1. It's timing must be predictable
2. It must be detectable by the animal

3. It must generate some form of physiological change that influences reproduction.

Pankhurst and Porter (2003) add that the cue might not have to be predictable if it signals an advantageous environmental change. Increasing temperature might fall into this category. Photoperiod is predictable, is capable of giving a reliable date cue, and would be expected to be more important at high latitudes where there is a greater difference in daylength with seasons (Pankhurst and Porter 2003). Temperature in many systems acts as a secondary cue after photoperiod, ensuring that reproduction happens at the most favourable time for offspring development. In cyprinid fish, increasing temperature of the water in spring leads to gonadal development (Stacey 1984). In some species (eg salmonids) nutritional status, in the form of energy reserves stored as fat, is also important and acts as a gating mechanism, which decides whether or not the fish can respond to the proximate stimuli such as photoperiod (Thorpe 1994). Social factors can also be important in many species (Pankurst 1995) such as the behaviour of conspecifics, courtship rituals and population density (Halliday 1974; Fleischack and Small 1978; Burmeister and Wilczynski 2005).

In the tropics, where conditions are more constant, other cues such as flooding or cloudiness (signaling the start of the rainy season) may trigger reproduction (Takemura et al. 2004). Because of the lack of large differences in photoperiod and temperature in the tropics, the relative importance of cues arising from the lunar cycle is greater in tropical and equatorial fishes (Takemura et al. 2004). Consequently, many families of reef fishes show lunar-related reproductive activity (Thresher 1984). Mating, spawning and release of eggs or larvae are all processes which can be linked to lunar phase (Omori 1995). There are various adaptive advantages to be gained from this:

1. Dispersal of larvae - spawning is timed to occur at high tides where currents can transport eggs and larvae to protect them from the heavily predated reef area (Robertson 1991).
2. Spawning success can be maximized by ensuring the largest number of animals is in reproductive condition and in one place at the same time and resulting in increased rates of mating and, therefore, fertilisation (Korringa 1957; Neuman 1975; Takemura et al. 2004).
3. Protection of adults, eggs, larvae or metamorphs by predator dilution or satiation (Darling 1938, Robertson 1991)

4. Protection of eggs or larvae in nursery sites (Korringa 1957, Christy 1978)

Points 2, 3 and 4 could also be applied to amphibians.

Reproductive synchrony is found in both animals and plants (Ims 1990).

The selective pressure can be on synchrony itself, where spawning or mating in temporal proximity maximises fitness, and is therefore selected for. Alternatively, reproduction during optimum conditions may be selected for, such as breeding during advantageous weather conditions, where the end result will be mating or spawning in synchrony but the selection pressure will have been different (Ims 1990).

Darling (1938) first proposed synchronization of reproduction as a predator swamping strategy, and it may protect offspring by confusing or satiating predators or predator dilution (reducing the risk of predation on any one individual by lowering the overall percentage of prey taken from the population), or varying combinations of these. Many fish species show adaptive lunar-synchronised reproduction, particularly in the tropics but also in temperate areas, although photoperiod and temperature can override or mask the effects of these cues at high latitudes (Figure 1).

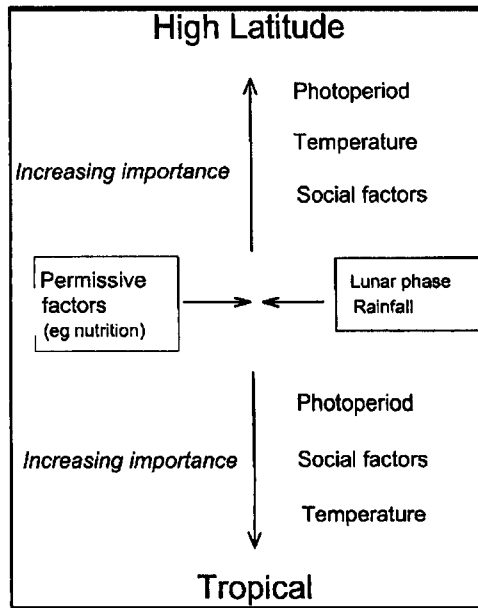


Figure 1. Environmental control of reproduction in fishes. From Pankhurst and Porter (2003).

Some of the findings on the environmental control of reproduction in fish can be applied to amphibians. It would be expected from considering fish that amphibians at high latitudes would be more affected by temperature, and that in tropical species lunar phase may be more important. However there are no studies that have tested this hypothesis. In amphibians, there may be additional permissive factors, such as age, as females of many species breed a year later than males (Berven 1990; Marvin 1996).

1.2.3 *Breeding Phenology in amphibians*

The precise timing of amphibian breeding depends on a number of endogenous and exogenous factors (Osseen and Wassersug 2002). Many differences in breeding phenology between sexes and between species are likely to be adaptive (Semlitsch 2008). The factors that affect amphibian migrations vary between sites and years (Timm et al. 2007), between geographic locations in the same species and between sexes, with males often arriving before females (Semlitsch 2008). Desiccation is a problem for many amphibians while migrating, due to their permeable skin making them susceptible to water loss (Shoemaker et al. 1992) and, as previously discussed, they are reliant on ambient temperature. This means that weather, particularly rainfall and temperature, is often important in determining amphibians' movements and most studies of amphibian breeding phenology have concentrated on these meteorological variables. Although amphibian reproduction is correlated with many environmental factors, it is important to be aware that this does not prove causation, particularly in cases where explanatory variables are highly correlated with each other such as barometric pressure, humidity and rainfall. There is little

experimental evidence of the kind needed to prove causation, although the correlations seen are evidence for possible causation.

There are several key reproductive parameters in the amphibian life cycle which can be considered when studying breeding phenology, for example:

1. The date the first (male or female) amphibian is sighted at the breeding pond,
2. The numbers of animals of each sex arriving each day,
3. The date that the first mating pair is observed,
4. The number of mating or amplexed pairs each day,
5. The date that the first spawn is laid,
6. The number of animals spawning each day,
7. In calling species, the date of onset and the duration of calling,
8. The number calling each day or an index of calling intensity,
9. The number of amphibians attending a breeding chorus on a given night.

I will now discuss some of the environmental cues which have been shown to be correlated with one or more aspects of amphibian breeding phenology.

Temperature

Because they are ectothermic, amphibians are dependent on ambient temperatures being high enough for physiological functioning and there is often a species-specific temperature threshold below which amphibians are not active (Table 1). Water temperature above a threshold is important for the physiological functioning of adults and also egg and larval development (Oseen and Wassersug 2002).

Table 1. Threshold temperatures for amphibian reproductive activity

Species	Reproductive parameter	Temperature threshold	Authors
<i>Rana sylvatica</i>	chorusing	>8-10°C	Howard (1980)
<i>Rana pipiens</i>	spawning	>8°C	Gilbert et al. (1994)
<i>Rana esculenta</i>	calling	>13-15°C (air) >12°C (water)	Obert (1975)
<i>Rana ridibunda</i>	calling	>13-15°C (air) >12°C (water)	Obert (1975)
<i>Alytes o. obstetricians</i>	calling	>7°C	Heinzman (1970)
<i>Bufo bufo</i>	breeding migration	>6°C	Reading (1998)
<i>Bufo bufo</i>	breeding migration	>4°C	Gittins (1980)
<i>Triturus cristatus</i>	breeding migration	>5°C	Verrell and Halliday (1985a)
<i>R. temporaria</i>	spawning	>3.1°C	Beattie 1985

Additionally, above these thresholds many species' day to day reproductive activity varies with water temperature, or air temperature (Table 2).

Table 2. Examples of amphibian reproduction affected by temperature

Species	Reproductive parameter	Explanatory variable	Authors
<i>Hyla arborea</i>	Duration of calling	Water temperature at 24.00h	Blankenhorn (1972)
<i>Bufo calamita</i>	Duration of calling	Water temperature at 24.00h	Blankenhorn (1972)
<i>Pseudacris crucifer</i>	Calling activity	Water temperature	Oseen and Wassersug (2002)
<i>Bufo americanus</i>	Calling activity	Water temperature 14-18°C	Oseen and Wassersug (2002)
<i>Rana clamitans</i>	Calling activity	Water temperature >22°C	Oseen and Wassersug (2002)
<i>Rana catesbeiana</i>	Calling activity	Water temperature >20°C	Oseen and Wassersug (2002)
<i>Pelobates cultripes</i>	Numbers active	Water temperature	Salvador and Carrascal (1990)
<i>Hyla arborea</i>	Numbers active	Water temperature	Salvador and Carrascal (1990)
<i>Bufo japonicus</i>	Breeding activity	Temperature	Okuno (1985)
<i>Bufo calamita</i>	Numbers active	Air temperature	Salvador and Carrascal (1990)
<i>Rana perezi</i>	Numbers active	Air temperature	Salvador and Carrascal (1990)
<i>Bufo quercus</i>	Movements	Maximum temperature	Greenberg and Tanner (2005)
<i>Triturus cristatus</i>	Breeding migration	Minimum temperature	Verrell and Halliday (1985a)

Rainfall and humidity

Rainfall (and also humidity, which is highly correlated with rainfall), is often important in constraining amphibian movements (Table 3) but the degree to which amphibians depend on rainfall varies with their life history characteristics, with some amphibians, particularly those with a terrestrial lifestyle, being less dependent on moisture. Some amphibians respond to barometric pressure, probably because drops in barometric pressure precede rainfall (Blankenhorn 1972, Oseen and Wassersug 2002). Low windspeed has been found to be necessary to stimulate calling in some species (e.g. *Rana catesbeiana* (Oseen and Wassersug 2002)) presumably because low windspeeds reduce desiccation risk.

Table 3. Amphibian reproductive activity and rainfall / humidity

Species	Reproductive parameter	Explanatory variable	Author
<i>Hyla arborea</i>	Onset of calling	Rain on previous day	Blankenhorn (1972)
<i>Bufo calamita</i>	Onset of calling	Rain on previous day	Blankenhorn (1972)
<i>Pseudacris crucifer</i>	Calling	Rainfall	Oseen and Wassersug (2002)
<i>Melanophryniscus rubriventris</i>	Breeding and spawning	Rainfall	Vaira (2005)
<i>Bufo terrestris</i>	Movements	Rainfall	Todd and Winne (2006)
<i>Ambystoma talpoidium</i>	Movements	Rainfall	Todd and Winne (2006)
<i>Rana sphenoccephala</i>	Movements	Rainfall	Todd and Winne (2006)
<i>Ambystoma tigrinum</i>	Movements	Rainfall	Todd and Winne (2006)
<i>Ambystoma m. columbianum.</i>	Migratory movements	Rainfall	Beneski et al (1986)
<i>Pelobates cultripes</i>	Number active	Rain on previous day	Salvador and Carrascal (1990)
<i>Bufo quercus</i>	Movements	Rainfall	Greenberg and Tanner (2005)
<i>Alytes cisternasii</i>	Mating activity	Rainfall	Marques (1992)
<i>Rana clamitans</i>	Calling	Humidity	Oseen and Wassersug (2002)
<i>Rana sylvatica</i>	Activity	Humidity	Bellis (1962)
<i>Hyperolius marmoratus</i>	Chorus attendance	Rainfall and Humidity	Henzi et al (1995)

Social facilitation

Social facilitation is often a factor influencing reproductive behaviour, particularly in callers. Often, the presence of calling males stimulates other males to call and, in many models of breeding behaviour, previous calling of conspecifics is a significant explanatory variable in a variety of species (Wells 1988, Brooke et al. 2000, Oseen and Wassersug 2002).

Seasonal and site-specific factors

Oseen and Wassersug's (2002) study showed that the combination of environmental variables responded to is different early and late in the season in the same species, between early and late breeders in the same pond and between different species at the same breeding site. For example, prolonged breeders respond to a different combination of environmental variables depending whether it is early or late in the breeding season and sympatric calling males of different species respond to different environmental cues even when calling together (Oseen and Wassersug 2002). For example, spring breeders such as *Rana sylvatica* and *Bufo americanus* responded primarily to time of day (they start calling at dusk),

but summer breeders such as *Rana clamitans* and *Rana catesbeiana* are stimulated to call by rising water temperature (Oseen and Wassersug 2002). Brooke et al (2000) looked at the influence of environmental variables on calling in the microhylid frog *Cophixalus ornatus* including temperature, rainfall, moon illumination, moon visibility, humidity and barometric pressure. They found that around 36% of the variation in intensity of calling activity was due to large scale factors which did not vary between sites such as weather, moon phase, or large-scale social facilitation, and around 64% was caused by site specific factors, such as microenvironmental factors or small scale social facilitation.

Once the effects of site and season were removed environmental factors accounted for little (<10%) of the variation in male calling. Brooke et al. (2000) suggested that large scale factors that affect all sites, such as rainfall, moon phase and temperature offer a window within which calling can take place but have less effect on day to day numbers of callers than small scale locally occurring factors, such as social facilitation (i.e. males calling because of other males calling).

Recently, the view that rainfall and temperature are the main factors that stimulate amphibians to breed has been challenged, and photoperiod has been suggested as a primary driver of reproductive behaviour in amphibians (Canavero and Arim 2009). The number of species calling at a site in Uruguay, over an 18 month period, was found to be highly correlated to increasing photoperiod. This is thought to be mediated by light stimulating melatonin release, a mechanism which has been shown to occur in amphibians (Jørgensen 1992; Bradshaw and Holzapfel 2007). Canavero and Arim (2009) suggest that amphibians' apparent response to temperature and rainfall could be due to these variables being highly reliant on time of year, and therefore strongly correlated with photoperiod.

Endogenous factors

The importance of rainfall and humidity seems to vary between species. Indeed, Oseen and Wassersug (2002) point out that, although rainfall and temperature can be good predictors of reproductive activity in amphibians, these variables alone do not explain the high degree of synchronisation that occurs in many explosive breeders. In some studies of anurans, particularly *B. bufo*, rainfall was not found to influence the arrival of toads at the

breeding site (Gittins 1983; Reading 1998). This has led several authors to conclude that there may be a large endogenous (i.e. internally arising) component to amphibian breeding phenology.

Indeed, it appears that there is only a certain percentage of amphibian reproductive activity that can be explained purely by environmental factors. For example, Sinsch (1988) found that the timing of *B. bufo* migration was fairly independent of climatic factors and concluded that endogenous components may make up a larger part of the variation in migration timing than is normally supposed. Wells (1979) made similar observations in the explosive breeding *Bufo typhonius* and suggested that an endogenous cycle interacts with an environmental cue in this species to produce highly synchronous breeding. Heinzmann (1970) found that the calling of the midwife toad *Alytes o. obstetricans* was not really affected by weather, and concluded that there was an endogenous cycle in these toads.

The examples above show that breeding phenology in amphibians seems to be influenced by many different factors, depending on species, mating system, geographical area, and also on site-specific factors. If any generalisation can be made about amphibian breeding phenology it is that

rainfall and temperature seem to be important for many, but not necessarily for all species and that their influence may also depend on other interacting variables. Temperature is clearly important as a threshold for physiological reasons, but its importance may be smaller once breeding has commenced, as long as it remains above the critical threshold. Similarly rainfall may not be quantitatively important, but some moisture is certainly needed to prevent desiccation.

1.2.4 *Lunar and tidal rhythms*

Animals, through evolution, have become adapted to predictable periodic cycles in the environment, caused by the solar and lunar cycles giving rise to seasons and tides, enabling them to be able to anticipate environmental changes, giving rise to adaptive behaviour such as spring reproduction (Sharma and Chandrashekar 2005). All animals show some kind of rhythm in their activity levels and other aspects of their behaviour.

Rhythms are present from the molecular to the whole animal level, so it is likely that they evolved early (Anders 1982). The most well known biological rhythm is probably the circadian (around a day) rhythm of 20-28h, but there are also longer rhythms which are known as infradian, and

include tidal, lunar, monthly, seasonal and yearly rhythms (Sharma and Chandrashekar 2005). The peak of a biological rhythm is known as the acrophase and is represented by the Greek letter θ . When endogenous periodic rhythms are established in a species, they continue even when the animal is isolated from its natural environment or held in constant conditions. Usually, these rhythms are entrained by an external factor, termed a Zeitgeber, of which light and temperature are the commonest (López-Olmeda et al. 2006). Many rhythms are controlled by the central nervous system and involve the hypothalamic – pituitary axis modulating the secretion of hormones such as gonadotrophins. For example in rabbitfish, which have a semilunar spawning rhythm, suppression of melatonin by moonlight leads to a rise in levels of reproductive hormones (Takemura et al. 2006).

It is well known that many marine animals have activity levels or reproductive behaviour that are synchronised with tidal cycles. The tidal cycle is the most obvious lunar cue in the seas, and is caused by the combined gravitational pull of the Sun and the Moon, which peaks twice per lunar month (at new moon and full moon). As well as gravitational changes, water depth, temperature, salinity, and pressure will change on a

cyclical basis and in the intertidal zone there will be a rhythm of exposure / immersion as well as wave action, all of which can be detected by animals (Morgan 2001). Animals that show lunar periodicity of behaviour fall into two categories, those that react directly to the lunar cue (e.g. incoming tide or increasing intensity of moonlight), and those that have an endogenous rhythm (Neumann 1981). Those without an endogenous rhythm can be misled by unexpected cues, for example, the red fronted lemur is active on moonlit nights, but ceases activity in a total lunar eclipse, indicating a direct response to lunar illumination (Donati 2001). Only where there is an endogenous rhythm are animals able to predict events. For example, marine iguanas (*Amblyrhynchus cristatus*) forage on algae in the intertidal zone at low tide. The animals start moving towards the foraging areas up to four hours before low tide, and their movements correspond more closely with predicted (tabulated) tide times, than with the actual tide-height and foraging area exposed, indicating an adaptive endogenous rhythm that is able to anticipate the times of the low tide (Wikelski and Hau 1995).

One way to differentiate between an endogenous rhythm and a direct response to some environmental cue is to remove the animal from its natural environment and keep it in constant conditions to see if the rhythm

continues. For example, the lunar synchronised gametogenesis and spawning in rabbitfish persists when the fish are held in tanks under constant (light and tidal) conditions, indicating a circalunar endogenous cycle (Rahman et al. 2000). However so-called constant conditions, although removing light and the effect of tides, do not remove the gravitational and geomagnetic effects of the moon so results must be interpreted with caution. Sometimes, one aspect of an animal's lunar-related behaviour can be endogenous and another component reactive. For example, emergence of the nocturnal lizard *Stenodactylus doriae* is controlled by an endogenous circalunar rhythm, demonstrated by the fact that lizards were still active during a lunar eclipse. However actual locomotor activity (number of tracks) decreased during the eclipse, suggesting a direct reaction to lunar light (Bouskila et al. 1993).

Many marine animals do not, in fact, respond to tides but to other cues from the moon, such as light. Many cues from the moon are found not only in the marine realm, but also in terrestrial and freshwater environments, such as the gravitational changes, lunar illumination, the length that the moon is visible per night and the polarisation of moonlight, as well as lunar modulated geomagnetic changes (Stolov 1965; Bell and Defouw 1966). It is

unsurprising then, that many lunar synchronised marine fauna do not respond to tidal cues *per se*, but directly to other cues from the lunar cycle, such as moonlight. Bentley et al (2001) provide evidence that many marine worms respond not just to tidal forces, but also to moonlight, and are able to detect very low light levels that mean they would easily be able to distinguish different moon phases. Hauenschild (1960) demonstrated that lunar light, not tides, is a Zeitgeber for synchronization of reproduction in *Platynereis dumerillii*, which is also temperature dependant (Goerke 1984). In rabbitfish, gonadal development, culminating in spawning, is synchronised to a species-specific lunar phase. This effect is thought to be mediated by moonlight intensity affecting melatonin production (Takemura et al 2004). Mummichog (*Fundulus heteroclitus*) exhibit semi-lunar synchronised spawning in habitats where there is no tidal stimulus (Hines et al. 1985).

As lunar cycles are complex, a brief discussion of them is relevant at this point. The lunar day is the time taken for the Moon to return to the same point in the night sky on consecutive days and is slightly longer than the solar day with duration of 24.8h, (this is called the circa-lunadian cycle). The Moon orbits the Earth in 27.3 days, which is known as the sidereal

month. This is the time taken for the moon to orbit with respect to the stars and is the moon's true orbital period. However, the Moon-Earth system moves in its orbit around the sun during this time. Therefore to return to the same phase the moon must travel more than 360 degrees around its orbit. The time taken for the moon to return to the same phase is, on average, 29.53 days, which is known as the synodic month (Figure 2).

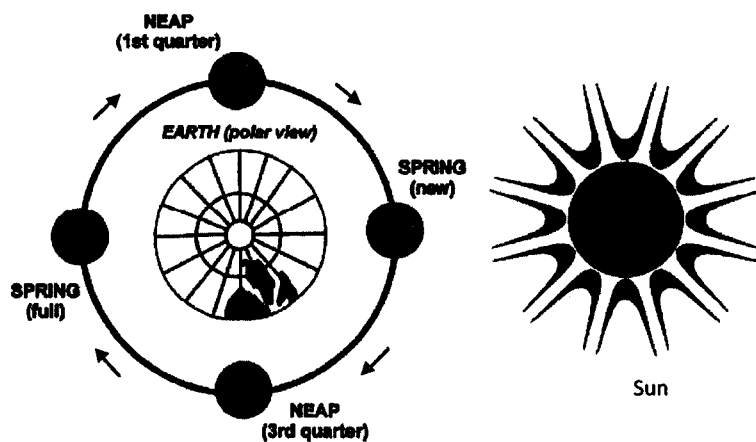


Figure 2. The lunar synodic cycle, showing the Moon orbiting the Earth; at new and full moons the Earth, Moon and Sun are aligned and at first and third quarter moons the Moon is at right angles to the Earth-Sun system. From Kvale et al. (1999).

The synodic lunar cycle is the most familiar to us, as the phases of the synodic month can be observed as the moon waxes and wanes. The moon orbits the earth, held in place by the gravitational attraction between them. The Moon, however, is also affected by the Sun's gravity which can affect the time taken for its orbit. For this reason, the figures given for the length of synodic and sidereal months is an average and can vary by as much as 12 and 7 hours respectively. At full and new moons, the Earth, Sun and Moon are aligned. This is termed lunar syzygy, and the combined gravity of Moon and Sun means that the strongest gravitational force affects the Earth at this time. The quarter moons, when the moon is at right angles to the Sun and the Earth, are termed quadrature and, at this point in the cycle, gravitational pull is at a minimum (Figure 3).











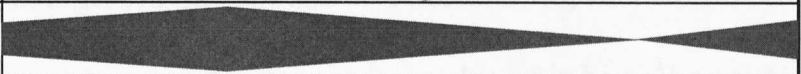
	Full moon	Waning gibbous	3rd quarter	Waning crescent	New moon	Waxing crescent	1st quarter	Waxing gibbous
Lunar phase	1	2	3	4	5	6	7	8
								
Days since full moon (DFM)	28,29, 0,1	2,3,4,5	6,7,8	9,10,11,12	13,14,15,16	17,18,19,20	21,22,23	24,25,26,27
N in category	4	4	3	4	4	4	3	4
Illumination								
Gravitational pull								
Geomagnetic fields								

Figure 3. Geomagnetic, illumination and gravitational changes caused by the lunar synodic cycle. The lunar effect on geomagnetism was adapted from Stolov (1965) and Bell and Defouw (1966).

Because the lunar orbit is an ellipse, the moon is not always at the same distance from the earth. The time when the moon is nearest the Earth is termed perigee, and when the moon is furthest from the Earth, apogee (Figure 4). The time taken for the Moon to move from apogee to perigee is 27.55 days and is known as the anomalistic month. The gravitational pull of the Moon on the earth is strongest at perigee.

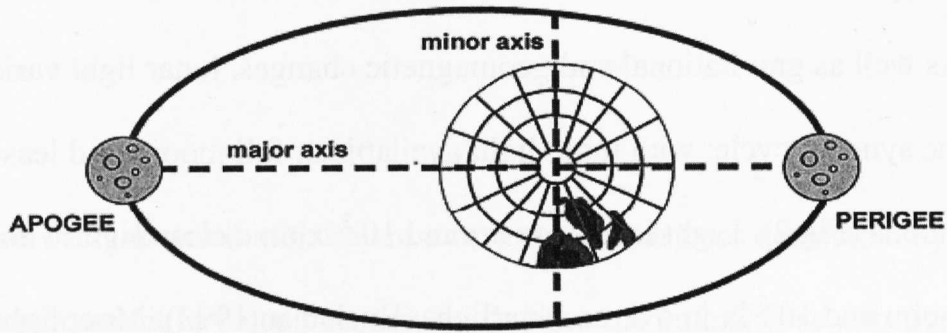


Figure 4. The anomalistic month. From Kvale et al. (1999).

The declination of the moon (its angle with respect to the Earth's equator) also affects its gravitational influence and the time taken for the moon to move from its maximum northerly declination to its maximum southerly declination is 27.32 days, which is known as the tropical month (Kvale et al 1999). The moon's departure from the ecliptic is important because the moon's influence on certain factors can be stronger when it is close to the ecliptic. For example, Stolov (1965) has shown that the moon affects the geomagnetic field of the Earth but only when the moon is within 3.5 degrees of the ecliptic plane. Geomagnetism is thought to vary with the synodic cycle, maximising in the third quarter, and minimising in the first quarter (Figure 3) (Stolov 1965; Bell and Defouw 1966).

As well as gravitational and geomagnetic changes, lunar light varies with the synodic cycle; with more light available at full moons and least at new moons (Fig.2). Light levels are around 10^{-3} lx on a clear night with a full moon and 10^{-5} lx in overcast starlight (Buchanan 1993). Moonlight is also brighter at perigee than at apogee. Because the lunar day is longer than the solar day the moon rises progressively later each day by approximately 50.5 minutes. A consequence of this is that at full moon, the Moon rises at dusk and sets at dawn, and at the new moon, the reverse is true; the Moon rises and sets at the same time as the Sun, hence the duration of moonlight as well as its brightness varies through the synodic cycle (McDowall, 1969). During the waxing moon, there is progressively more light available in the first half of the night, culminating at the full moon which rises as the sun is setting and remains until dawn. In the second half of the lunar cycle, the second half of the night is the lighter part culminating in the new moon, where the moon rises at dawn. It is clear that not only the brightness of lunar light changes, but also the type and duration of ecological niche available changes on a cyclical basis (Morgan, 2001). There are also other, longer term lunar cycles, but a discussion of these is beyond the scope of

this thesis, which is mainly concerned with the 29.53 day lunar synodic cycle.

It has been shown that there are a variety of lunar cues detectable by animals, and it has also been shown that marine animals use non-tidal cues from the moon. It is then, logical to expect that these cues can also be detected by terrestrial and freshwater animals and, in cases where this may be adaptive, aspects of their behaviour can be linked to lunar cycles.

Indeed, terrestrial taxa often exhibit lunar periodicity and the phenomenon exists across diverse phyla. Lunar periodicity is particularly common in insects (particularly Lepidoptera), mammals (especially bats and rodents) and birds. Recently, lunar-related behaviour has been reported in a variety of other terrestrial species including the badger (*Meles meles*)(Dixon et al. 2006) and elephants (Barnes et al. 2006). There is also a large body of research on a lunar effect in humans (*Homo sapiens*), which is often confounded by folklore and cultural factors, and provides no firm evidence that humans are affected by lunar cycles (Foster and Roenneberg 2008).

1.2.5 *Lunar rhythms in amphibians*

The regular environmental changes produced by the lunar cycle satisfy Sumpter's (1990) criteria for environmental control of reproduction. For example, moonlight varies with a regular and predictable pattern, amphibians' eyes are capable of detecting low levels of illumination (Cornell and Haliman 1984; Buchanan 1998) and light from the moon has been shown to directly stimulate reproductive hormones in fish and other animals (Takemura 2004). Geomagnetism has been shown to similarly vary in a predictable way with lunar phase and can be detected by amphibians (Phillips 1986a, b; Phillips et al. 1995)) although how this could be translated into reproductive behaviour is unclear.

There are several examples in the literature of amphibian activity being affected by lunar phase (e.g. Ferguson 1960, Church 1960a, b, 1961, Fitzgerald and Bider 1974, Tuttle and Ryan 1982, Byrne 2002, Byrne and Roberts 2004, Deeming 2008) which are discussed in more detail in Chapter 3, where I carry out a review of lunar-related behaviour in amphibians. Most studies of amphibian reproductive phenology, however, do not take moon phase into account and there has been little written about

this in recent years. Knowing which environmental variables affect amphibians and in which way is important in their conservation. For example, knowing when they are likely to be active could aid in the design of efforts to prevent road kill, which can wipe out 20% or more of an amphibian population in a single breeding season (Timm et al. 2007; Hels and Buchwald 2001).

Paton and Crouch (2001) suggest that more amphibian phenology studies are needed in different areas, in order to develop conservation strategies. Previous studies of mass movements of amphibians or breeding phenology have over-emphasised climatic variables and have often not taken lunar and geomagnetic changes into account. Considering lunar phases is particularly important when monitoring amphibian populations over a number of years. As the lunar synodic cycle is 29.53 days and the length of calendar months vary from 28-31 days, the synodic cycle is out of phase with calendar months and the dates of full moons occur on a different Julian dates each year. If monitoring is carried out on the same Julian date each year, the number of amphibians active could fluctuate depending on whether the survey was carried out around the full or new moon giving misleading results about the actual size of breeding populations.

It is clear, from the literature reviewed here, that that most studies of breeding phenology concentrate on weather variables. While it is recognised that amphibians undoubtedly respond to weather, this is only part of the picture. Amphibians are also sensitive to other environmental stimuli, which may affect behaviour and reproduction that are not often considered, such as lunar illumination, geomagnetic and gravitational changes, electrical and seismic activity. The pineal gland of amphibians is light-sensitive, which is important in regulating the circadian and other biological rhythms (Beebee and Griffiths 2000). Most amphibians are nocturnal and have dark-adapted eyes which are capable of detecting very low light levels and changes in intensity corresponding to different lunar phases. However, very few studies of factors affecting amphibian migrations have considered moon phase as a variable.

There are also other non-meteorological variables that are understudied in amphibian ecology. Many amphibians, but particularly newts, are able to sense and respond to electrical activity in water (Ranta 1990) and, as previously discussed, amphibians are sensitive to geomagnetism. The effects of geomagnetic storms on breeding migrations will be considered in

Chapter 6. Amphibians can also detect seismicity (Koyama et al. 1982; Lewis and Narins 1985), and the effect of a large earthquake on amphibian breeding behaviour is considered in Chapter 4. Amphibians are also sensitive to changes in water chemistry (Vitt et al. 1990).

1.3. The hypotheses to be tested

The present study seeks to make a novel contribution to what is known about the effects of geophysical variables on amphibian reproductive behaviour. I have concentrated particularly on lunar cycles, but I also considered other geophysical aspects that could affect amphibian breeding phenology, such as seismic and geomagnetic activity. While carrying out fieldwork, I was fortuitously able to observe amphibians' response to a large seismic event, so I have included a chapter on the effect of earthquakes on amphibian activity during the breeding season. As both seismic activity and lunar phase affect the earth's magnetic field, and amphibians (particularly urodeles) are sensitive to small geomagnetic changes, I have also considered geomagnetism. The factors that affect amphibian breeding are likely to vary with the ecology and mating system of the species in question, so I have attempted to relate amphibians'

responses to their possible adaptive function. Finally, I have attempted to model amphibian breeding phenology using statistical models, and to use these in a predictive way.

Chapter 2: The effects of lunar phase on amphibian reproduction

This chapter has also been published as Grant, R.A., Chadwick, E.A. and Halliday, T.R (2009) The lunar cycle: a cue for amphibian reproductive phenology? *Animal Behaviour* 78(2): 349-357.

2.1 Introduction

The position of the Moon in relation to the Earth and the Sun gives rise to several cycles that occur at regular intervals. The lunar synodic cycle (full moon to full moon) has an average length of 29.5 days and causes a number of environmental changes that can be perceived by animals, such as the brightness of lunar light and gravitational changes, with maximum gravitational pull occurring when the Moon and Sun are aligned (at periods of new and full moons). Geomagnetic fields are also modulated by the lunar cycle, and these changes can be detected and are used as navigational or temporal cues by animals (Phillips 1986b; Lohmann and Willows 1987; Fischer et al. 2001).

Many marine animals take advantage of cues from the 29.5-day lunar cycle to synchronize their reproduction. This may protect adults, eggs and larvae from predation (by diluting predation risk or by using tidal currents to disperse or protect larvae), or maximize spawning success by ensuring the largest number of animals are in reproductive condition and in one place at the same time (Takemura et al. 2004). Lunar periodicity in marine animals is not necessarily tidally mediated; some marine animals respond specifically to lunar light (Takemura et al. 2004). Many fish show lunar synchronization which is not influenced directly by tides, for example, rabbitfish, *Siganus guttatus*, held in tanks continue to spawn at a species-specific lunar phase (Rahman et al. 2000). Mummichog, *Fundulus heteroclitus*, show semilunar synchronized spawning in habitats where there is no tidal stimulus (Hines et al. 1985). In addition to marine animals, various terrestrial birds and mammals have reproductive cycles linked to lunar phase (Cowgill et al. 1962; Erkert 1974; Archibald 1976; Dixon et al. 2006). Lunar periodicity of reproduction has also been reported in amphibians. The Javanese toad, *Bufo melanostictus*, ovulates on or near a full moon (Church 1960a) and lunar phase significantly affects both frequency of mating and activity level in the frog *Crinia georgiana* (Byrne 2002). Fitzgerald and Bider (1974) reported reduced locomotory (but not

necessarily reproductive) activity in the toad *Bufo americanus* during full moon phases. In anuran species with short breeding seasons, often called explosive breeders (Wells 1977, 2007), females arrive at the breeding site synchronously, either at the same time as or shortly after males, which actively compete for females. In contrast, prolonged breeders are characterized by males calling to attract females, often defending territories (Wells 1977, 2007). Explosive and prolonged breeding represent two extremes along a continuum of mating strategies. Although rainfall and temperature can be good predictors of reproductive activity in amphibians (Oseen and Wassersug 2002) these variables alone do not explain the high degree of synchronization that occurs in explosive breeders. In some studies of anurans, particularly *B. bufo*, rainfall was not found to influence the arrival of toads at the breeding site (Gittins 1983; Reading 1998). Indeed, this has led several authors to conclude that there may be a large endogenous component to amphibian breeding phenology. Sinsch (1988) found that the timing of *Bufo bufo* migration was fairly independent of climatic factors and concluded that endogenous components may make up a larger part of the variation in migration timing than normally supposed. Wells (1979) made similar observations in the explosive breeding *Bufo typhonius* and suggested that an endogenous cycle interacts with an

environmental cue in this species to produce highly synchronous breeding. Moon phase is an often overlooked environmental cue that could affect reproductive timing, and in this study we tested the hypothesis that periodicity in the breeding phenology of amphibians reflects periodicity in the lunar cycle. We analysed various parameters related to reproductive activity for a range of species, sites and years. For anurans, these parameters included (1) large arrival events (days on which more than 10% of the total in each year arrived), (2) large amplexus events (days on which more than 2% of the annual total number of mating pairs were observed), (3) large spawning events (days on which more than 10% of the annual total of spawning individuals were observed) and (4) first evidence of spawn. For urodeles, parameters included (1) first sighting, (2) peak (mode) arrival dates, and (3) median departure dates.

2.2 Methods

2.2.1 General Methods

We collected data from three sites: San Ruffino Lake, Italy, Llandrindod Wells, U.K. and Marston Pond, U.K. In all cases, individuals were located

by sight during transects surveyed on foot using powerful torches (flashlights). In all three surveys the torches used were considerably brighter than the ambient moonlight, minimizing bias caused by animals being more visible on a full moon. At San Ruffino Lake the torch used was an Energizer Halogen, at Llandrindod Wells a Clulite CL1, and at Marston Pond an Ever-Ready, all of which had a brightness between 5×10^3 and 5×10^6 lx. For comparison, light levels are around 10^{-3} lx on a clear night with a full moon and 10^{-5} lx in overcast starlight (Buchanan 1993). At all three study sites, we noted little or no deviation in amphibian movement towards the water on the approach of surveyors or illumination. The San Ruffino site is in a rural and undeveloped area and the part of the lake that was surveyed is free from artificial illumination. Llandrindod Wells Lake is in a semirural area, and Marston Pond is in an urban area, where artificial lighting is higher. Unfortunately, as the data were analysed retrospectively, the level of artificial light at the sites was not quantified.

2.2.2 Study Sites and Site-specific Methods

San Ruffino Lake

San Ruffino Lake, Monte San Martino, Italy (43°00'4"N, 13°23'4" E) supports large breeding populations of *B. bufo*, and smaller numbers of *Rana klepton hispanica* living in mixed populations with *Rana bergeri* (klepton indicating that this frog is a fertile hybrid). *Rana bergeri* and *R. kl. hispanica* were treated as a single species in this study as they are virtually impossible to distinguish in the field, being morphologically almost identical (Vignoli et al. 2007), and are referred to as *R. bergeri* below. Treefrogs, *Hyla intermedia*, were also present at the site but were excluded from analysis because of the small numbers encountered on land. A transect 2.5 km long on one side of the lake was surveyed once by R.G. each evening at dusk during the breeding season (April to June), for 40 nights in 2006 and 44 nights in 2007. The time taken to survey the transect was approximately 1.5 h. To reach the breeding site, the amphibians had to cross a pale-coloured lakeside path approximately 1.5 m in width, on which the animals could be clearly seen. Individuals were difficult to see as they entered the undergrowth at the side of the path, so only animals crossing the

path were counted, thereby minimizing differences in detection probability caused by the changing intensity of lunar light. Individuals on land around the perimeter of the lake were counted as they moved towards the water and categorized by species and as single or amplexed in 2006, and additionally by sex in 2007.

Marston pond

Marston Pond, Oxford, U.K. (51°46.2' N, 1°14.10' W) supported breeding populations of *B. bufo* and *Rana temporaria*, but may no longer be an amphibian breeding site. The circumference of the pond was patrolled each night for around 2 h from dusk by T.R.H. during the breeding season (March and April) from 1978 to 1987 (*B. bufo*) and 1979–1985 (*R. temporaria*) with the exception of 1984. As the pond was shaded by trees and bushes, the moon was not bright enough to detect animals without artificial illumination, so it is unlikely that the changing moonlight intensity biased the results.

Numbers of males and females arriving at the breeding site (on land) and the number of spawning pairs (in water) of *B. bufo* and *R. temporaria* were

recorded. The date of the first spawn observed was also recorded from 1977 to 1987 (*B. bufo*) and in 1977 and 1980–1984 (*R. temporaria*). The length of the breeding season varied between 8 and 42 days for *B. bufo*, and between 18 and 23 days for *R. temporaria*.

Llandrindod Wells Lake

Llandrindod Wells Lake, Wales, U.K. (52°14' 0" N, 3° 22' 8"W) supports a large population of *B. bufo*. A complete circuit of the lake, 1 km in circumference, was walked each night of the breeding season (March and April) in 2000 and 2001 by E.A.C. and repeated every half hour from dusk until arrival numbers declined for three consecutive circuits, to below 20 individuals for at least two circuits and below 10 for the final circuit.

Numbers of single males, females and amplexed pairs were recorded. The time taken for the survey was variable but was typically 2 h from dusk.

Animals were clearly visible as they crossed a wide metalled path or a road surrounding the lake, so changes in moonlight intensity were unlikely to have influenced detection probability at this site. The length of the breeding season was 55 days in 2000 and 49 days in 2001.

2.2.3 Literature Search

Data on significant reproductive events, such as the date on which the first amphibian was sighted or the date on which the first spawn was laid, are widely available in published literature. Data on reproductive events were obtained from various research papers, and previously unpublished data on newt arrivals were supplied by E.A.C. (for details of data collection methods, see Chadwick et al. 2006). The criteria for inclusion of published data were as follows: the study showed clearly either consecutive daily arrivals for each year, or the dates of significant reproductive events over a number of years, either as raw data or in a form that could be easily estimated with a reasonable degree of accuracy (i.e. to within 2–3 days). We excluded data that showed weekly or monthly arrivals totals, that combined arrival and departure totals, or that averaged totals over a number of years, and data where the exact dates of the study were not given. Five published studies were used involving three urodele and six anuran species. Table 4 shows a numbered list of all data sources used in this study; these are referred to by study number in the following analyses.

Table 4. The source of data used in this investigation

Study number	Source	Period	Site	Species	Data used
1	Unpublished R.G.*	2006–2007	San Ruffino, Italy	<i>Rana bergeri</i>	N arriving
1	Unpublished R.G.*	2006–2007	San Ruffino, Italy	<i>Bufo bufo</i>	N arriving
2	Unpublished T.R.H.*	1978–1987	Oxford, England	<i>Bufo bufo</i>	N arriving
2	Unpublished T.R.H.*	1978–1987	Oxford, England	<i>Rana temporaria</i>	N arriving
2	Unpublished T.R.H.*	1978–1989	Oxford, England	<i>Bufo bufo</i>	First spawn
2	Unpublished T.R.H.*	1979–1983	Oxford, England	<i>Rana temporaria</i>	First spawn
3	Unpublished E.A.C.*	2000–2001	Llandrindod Wells, Wales	<i>Bufo bufo</i>	N arriving
4	Blaustein et al. 2001	1982–1989	Oregon, U.S.A.	<i>Rana cascadae</i>	First spawn
4	Blaustein et al. 2001	1982–1989	Oregon, U.S.A.	<i>Bufo boreas</i>	First spawn
5	Beebee 1995	1978–1994	Sussex, England	<i>Rana temporaria</i>	First spawn
5	Beebee 1995	1978–1994	Sussex, England	<i>Bufo bufo</i>	First spawn
5	Beebee 1995	1978–1994	Sussex, England	<i>Rana esculenta</i>	First spawn
5	Beebee 1995	1978–1994	Hampshire, England	<i>Bufo calamita</i>	First spawn
5	Beebee 1995	1978–1994	Sussex, England	<i>Lissotriton vulgaris</i>	First sighting
5	Beebee 1995	1978–1994	Sussex, England	<i>Lissotriton helveticus</i>	First sighting
5	Beebee 1995	1978–1994	Sussex, England	<i>Triturus cristatus</i>	First sighting
6	Tryjanowski et al. 2003	1978–2002	Western Poland	<i>Rana temporaria</i>	First spawn
6	Tryjanowski et al. 2003	1978–2003	Western Poland	<i>Bufo bufo</i>	First spawn
7	Unpublished E.A.C.	1981–1987, 1997–2005	Llysdinam, Wales	<i>Lissotriton vulgaris</i>	N arriving
7	Unpublished E.A.C.	1981–1987, 1997–2005	Llysdinam, Wales	<i>Lissotriton helveticus</i>	N arriving
7	Chadwick et al. 2006	1981–1987, 1997–2005	Llysdinam, Wales	<i>Lissotriton vulgaris</i>	Median depart
7	Chadwick et al. 2006	1981–1987, 1997–2005	Llysdinam, Wales	<i>Lissotriton helveticus</i>	Median depart
8	Unpublished E.A.C.†	1981–1987, 1997–2005	Llysdinam, Wales	<i>Lissotriton vulgaris</i>	Peak arrival
8	Unpublished E.A.C.†	1981–1987, 1997–2005	Llysdinam, Wales	<i>Lissotriton helveticus</i>	Peak arrival
9	Gittins et al. 1980	1978	Llandrindod, Wales	<i>Bufo bufo</i>	N arriving

Studies were carried out by R. A. Grant (R. G.), T. R. Halliday (T. R. H.) and E. A. Chadwick (E. A. C.).

* See text for details of methods.

† See Chadwick et al. (2006) for details of methods.

2.2.4 Study Species and Breeding Biology

Of the anuran species studied here, *Bufo boreas*, *B. bufo*, *Rana cascadae* and *R. temporaria* are explosive breeders, where males engage in scramble competition for females, the sex ratio is male biased and breeding is highly synchronized (Beebee and Griffiths 2000; Blaustein et al. 2001). *Bufo*

calamita has a more prolonged breeding season, and calls to attract females (Beebee and Griffiths 2000). *Rana bergeri* and *R. kl. hispanica* are mainly aquatic frogs that are often found in mixed populations of which *R. bergeri* is usually the most numerous, *R. kl. hispanica* being a hybridogenetic frog, which depends on *R. bergeri* for its reproduction (Lanza et al. 2007). *Rana esculenta* is also a highly aquatic pool frog. The three newt species included in this study (*Lissotriton* [previously *Triturus*] *vulgaris*, *L. helveticus* and *Triturus cristatus*) were all studied in the U.K. where breeding migrations occur over a protracted period between January and April (Beebee and Griffiths 2000). The three newts studied here often share the same habitat. All the species studied here are spring breeders and the literature suggests that emergence from overwintering sites and breeding migrations are largely constrained by climate.

Geographical variation in climate can cause considerable variation in breeding phenology. For *R. cascadae* and *B. boreas*, emergence occurs following snowmelt (Corn 2003) and other species seem to be largely constrained by temperature (Reading 1998). Breeding migrations vary considerably in terms of distance travelled. The pool frogs *R. bergeri* and *R. esculenta* hibernate in the water and *B. calamita* and *R. cascadae*

overwinter at or adjacent to the breeding sites, so these species do not make extensive breeding migrations (Beebee and Griffiths 2000). Some individuals of *R. temporaria* overwinter in the pond and others migrate (Verrell and Halliday 1985b). However, *B. bufo* is largely terrestrial and may overwinter in refugia up to 1200 m from the breeding site (Sinsch 1988).

2.2.5 Moon Phase Data

We obtained the dates of full moons from the US Naval Observatory Astronomical Applications Department (<http://aa.usno.navy.mil/data/docs/MoonFraction.php>) for midnight at Universal Time (GMT). Moon phase is independent of the geographical position of the observer. No account was taken of the actual light levels at the breeding sites, moon rise or set times, the moon's angle of declination, other lunar cycles (such as the 18.6-year nodal cycle which may affect biota, (Archibald 1977)) or whether or not the moon was visible on a given night. This was because most of the data were collected before the current hypothesis was proposed.

2.2.6 *Derived Data*

For each of the recorded reproductive ‘events’ (e.g. first spawn or sighting), we counted the days since full moon (DFM) and thereby assigned DFM values from 0 to 29 to each date, where 0 represents the full moon. To facilitate graphical presentation of the data, the DFM values were grouped into eight lunar phases (Figure 3), which we used to plot circular histograms, with the length of the bar representing the frequency of reproductive events occurring (see Results). The circular histograms show the actual frequencies of reproductive events and therefore the scales vary depending on the value of N in each case. The number of anuran arrivals recorded at the breeding sites on each day was expressed as a percentage of the total observed population for each species, in each year and for each site (% arrivals), so that data from different years could be pooled. We assigned DFM values to dates on which more than 10% of the total arrived, more than 10% of males or females arrived and more than 10% of the total were spawning (Marston Pond only), and the dates on which more than 2% of the total amplexed pairs were observed at the other two sites.

2.2.7 Statistical Analysis

We analysed the data for correlation with lunar phase. Most of the data were collected before the current hypothesis was proposed, thereby reducing the likelihood of observer bias. We converted DFM values to angles ($^{\circ}$) by dividing by 29.5 (the length, in days, of the lunar cycle) and then multiplying by 360° so that the data could be analysed using circular statistics. To assess whether reproductive events showed lunar periodicity, we analysed data using Rao's spacing test (Batschelet 1981), unless otherwise specified. Rao's spacing test is more powerful and robust than many other circular goodness-of-fit-tests, and is able to analyse bimodal and multimodal distributions, whereas other tests, such as the Rayleigh test and Watson's U_2 are not (Bergin 1991). Rao's spacing test is robust even at small sample sizes, but also shows a low frequency of type I errors when analysing data that show no pattern. The only cases where Rao's spacing test fails are in distributions that show directional avoidance (Bergin 1991). Where data appeared to be unimodal, we also used the Rayleigh test for departure from randomness, and where this produced a different outcome from Rao's test, the Rayleigh test statistic is also presented. The null hypothesis that reproductive events would be equally or randomly spaced

throughout the lunar cycle was tested for each data set. All data were analysed using the circular statistics software Oriana 2.0 (<http://www.kovcomp.co.uk/oriana/>, Kovach Computing Services, Anglesey, U.K.). Oriana 2.0 does not give exact P values for Rao's spacing test, but provides the test statistic U. For nonsignificant results Oriana gives P as a range; this is presented as provided. For significant P values Oriana uses the form $P < 0.05$ or $P < 0.01$. To find more precise values for significant results, we used an expanded table of P values for Rao's spacing test (Russell and Levitin 1995), which includes P values greater than 0.001 for $U = 1-220$. Where P is given for a range of U we take the conservative approach of rounding U values down; this potentially overestimates P and therefore provides a conservative estimate of significance. The sample sizes in this study consist of the number of days on which the reproductive event (e.g. large spawn event, large arrival event) occurred; however, the actual sampling units are individuals, or pairs of animals. While this could be statistically problematic, the fact that on all days more than one individual or pair arrived means that the bias in this case would be towards type II error, and the significant results observed in this study can be deemed trustworthy. Another possible source of statistical error may come about through multiple testing (when there are a large number of tests, some will

reach significance from chance alone (Cross and Chaffin 1982)); this is discussed below.

2.3 Results

2.3.1 Large Arrival Events

For most anurans tested large arrival events occurred around the full moon (studies 1,2,3,9; Table 4, Figure 5). Large arrival events of *B. bufo* at Marston Pond occurred primarily in phases 1 and 8 (around the full moon; males: $U = 171$, $N = 40$, $P = 0.003$; females: $U = 188$, $N = 44$, $P < 0.001$). At Llandrindod Wells, all *B. bufo* large arrival events occurred in phases 6–8 (the waxing to full moon; males: $U = 226$, $N = 5$, $P = 0.006$; but note $U > 220$ so P is overestimated; females: $U = 202$, $N = 6$, $P = 0.016$). At San Ruffino, the two large *B. bufo* arrival events occurred on days 0 and 1 of the lunar cycle (full moon, phase 1). The three large arrivals of *R. bergeri* occurred on days 0, 3 and 5 of the lunar cycle (on, and just after, the full moon, phases 1 and 2), but no statistical tests were carried out owing to the small sample size. Conversely, *R. temporaria* arrivals were uniformly

spaced with respect to moon phase (males: $U = 118$, $N = 15$, $P = 0.5-0.9$;
 females: $U = 125$, $N = 25$, $P = 0.1-0.5$).

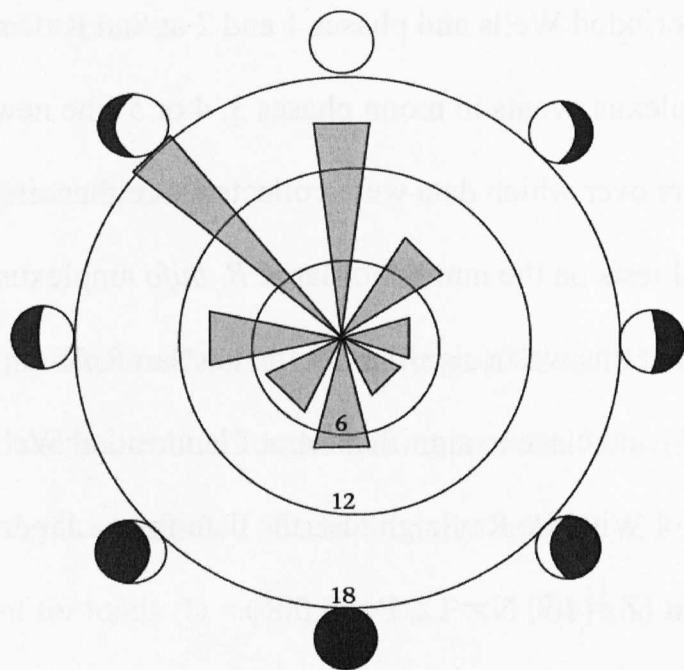


Figure 5. Large arrival events: frequency of occurrence in each moon phase, for *B. bufo*, *R. temporaria* and *R. bergeri* at Marston Pond, San Ruffino Lake and Llandrindod Wells Lake. See Figure 3 for moon phases.

2.3.2 Large Amplexus Events

Amplexed pairs at San Ruffino Lake and Llandrindod Wells Lake were seen more frequently around the time of the full moon (studies 1,3; Table 4). When total numbers of amplexed pairs at the sites were counted, 90% of those recorded at Llandrindod Wells Lake, and 84% of those recorded at

San Ruffino Lake occurred in phases 1, 2, 7 and 8 (around the full moon). Most large amplexus events ($>2\%$ of the total) occurred in phases 1, 7 and 8 at Llandrindod Wells and phases 1 and 2 at San Ruffino. There were no large amplexus events in moon phases 3, 4 or 5 (the new moon) in any of the 4 years over which data were collected, at either site (Figure 6).

Statistical tests on the number of large *B. bufo* amplexus events occurring showed that this was a significant effect at San Ruffino, ($U = 233$, $N = 12$, $P < 0.001$) and close to significance at Llandrindod Wells ($U = 160$, $N = 12$, $P = 0.096$). With the Rayleigh test, the data from Llandrindod Wells were significant ($Z = 4.9$, $N = 12$, $P = 0.005$).

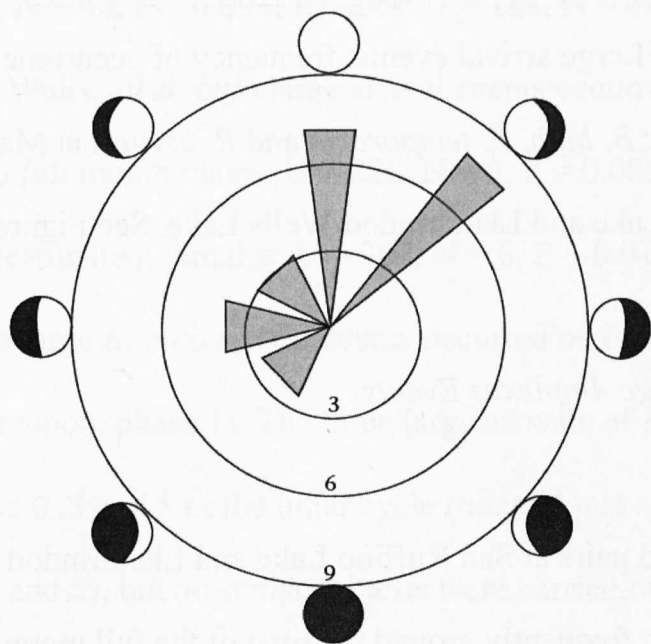


Figure 6. Large amplexus events: frequency of occurrence in each moon phase for *B. bufo* at Llandrindod Wells Lake and San Ruffino Lake. See Figure 3 for moon phases.

2.3.3 Large Spawning Events

Large spawning events at Marston Pond from 1978 to 1987 occurred more frequently around and soon after the full moon, with 75% of large spawning events occurring in phases 8, 1, 2 and 3 (study 2; Table 4, Figure 7). This was significant for toads ($U = 180, N = 38, P < 0.001$) and for frogs ($U = 184, N = 12, P = 0.02$).

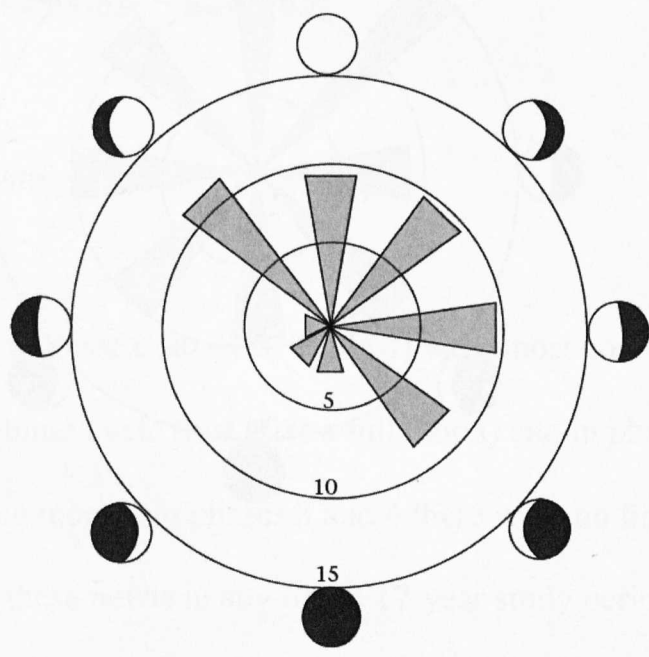


Figure 7. Large spawning events: frequency of occurrence in each moon phase for *B. bufo* and *R. temporaria* at Marston Pond. See Figure 3 for moon phases.

2.3.4 First Spawn

First spawn dates occurred uniformly throughout the lunar month (studies 2,4,5,6; Table 4, Figure 8). Table 5 shows the statistical outcomes of tests on the first spawn data.

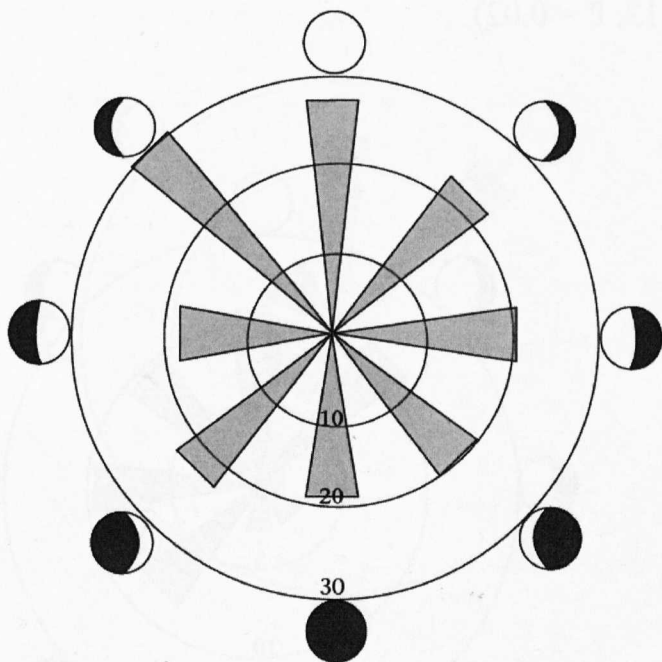


Figure 8. First spawn: frequency of occurrence in each moon phase for six anuran species at eight sites, as listed in Table 2. See Figure 3 for moon phases

Table 5. Statistical outcomes for first spawn data, showing no significant outcomes

Species and location	N	Rao's spacing test (U)
<i>Rana temporaria</i> , western Poland	18	102***
<i>Bufo bufo</i> , western Poland	18	117**
<i>Bufo boreas</i> , Lost Lake, Oregon, U.S.A.	14	113**
<i>Rana cascadae</i> , Site 1, Oregon, U.S.A.	14	133*
<i>Bufo boreas</i> , Three Creeks Lake, Oregon, U.S.A.	15	113**
<i>Bufo boreas</i> , Todd Lake, Oregon, U.S.A.	15	137*
<i>Rana cascadae</i> , Todd Lake, Oregon, U.S.A.	16	121**
<i>Bufo bufo</i> , Marston Pond, U.K.	10	113**
<i>Rana temporaria</i> , Marston Pond, U.K.	6	82**
<i>Rana esculenta</i> , Sussex, U.K.	16	154*
<i>Rana temporaria</i> , Sussex, U.K.	14	130*
<i>Bufo calamita</i> , Hampshire, U.K.	16	146*

*P = 0.1–0.5; **P = 0.5–0.9; ***P = 0.9–0.95.

2.3.5 First Sighting

First sightings in Sussex (study 5; Table 4) were most commonly made in phase 2 of the lunar cycle (just after a full moon) and in phases 5–8 (the new and waxing moon). In phases 3 and 4 there were no first sightings of any species of these newts in any of the 17-year study period (Figure 9).

This difference was significant for *L. helveticus* ($U = 186$, $N = 16$, $P = 0.006$), but not for *T. cristatus* ($U = 150$, $N = 17$, $P = 0.1-0.5$) or *L. vulgaris* ($U = 144$, $N = 16$, $P = 0.1-0.5$).

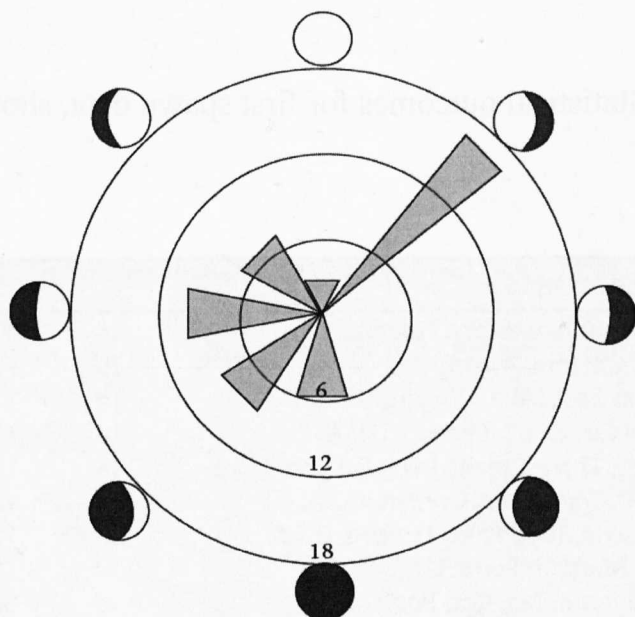


Figure 9. First sighting: frequency of occurrence in each moon phase for *L. vulgaris*, *L. helveticus* and *T. cristatus* in Sussex, U.K. See Figure 3 for moon phases.

2.3.6 Peak (Mode) Arrivals

Peak (mode) arrival dates at Llysddinam Pond (study 8; Table 4) occurred most frequently around the full moon (phase 1) and the new moon (phases 5 and 6). Few peak arrival dates occurred during the moon's third quarter

(phase 3) (Figure 10). When the modal values of males and females were analysed together for *L. vulgaris* the outcome was significant ($U = 168$, $N = 30$, $P = 0.017$); however, when the sexes were analysed separately the outcome was not significant for males ($U = 125$, $N = 15$, $P = 0.5-0.9$) or females ($U = 131$, $N = 15$, $P = 0.1-0.5$). For *L. helveticus* the outcome was not significant, whether analysed together or separately (males: $U = 137$, $N = 15$, $P = 0.1-0.5$; females: $U = 119$, $N = 15$, $P = 0.5-0.9$).

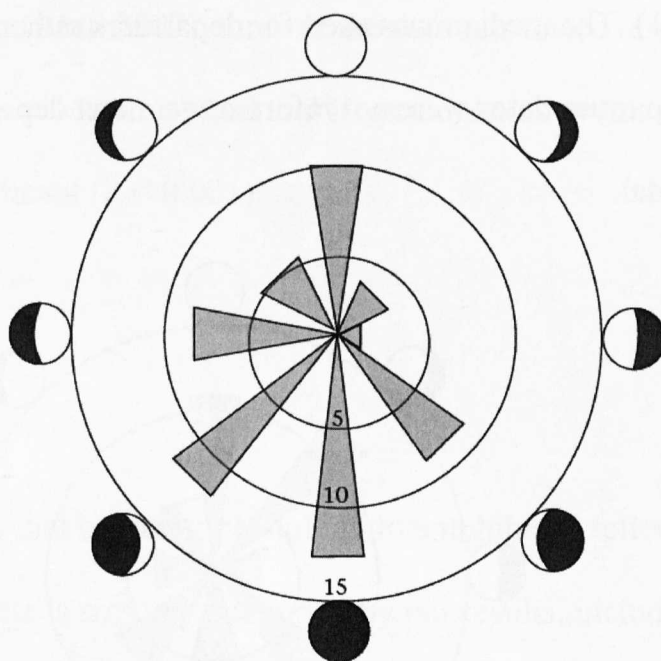


Figure 10. Peak (mode) arrival: frequency of occurrence in each moon phase for *L. vulgaris* and *L. helveticus* at Llysddinam pond, Wales, U.K. See Figure 3 for moon phases.

2.3.7 Departure Dates

There was no significant difference in the frequency of median departure dates from Llysdinam Pond occurring in each moon phase for the two newt species studied (*L. helveticus*: males: $U = 107$, $N = 15$, $P = 0.5-0.9$; females: $U = 96$, $N = 15$, $P = 0.9-0.95$; *L. vulgaris*: males: $U = 119$, $N = 12$, $P = 0.5-0.9$; females: $U = 125$, $N = 12$, $P = 0.5-0.9$; study 7; Table 4, Figure 11). The median was used for departures rather than the mode, as mode departure dates were not informative, newt departures being highly multimodal.

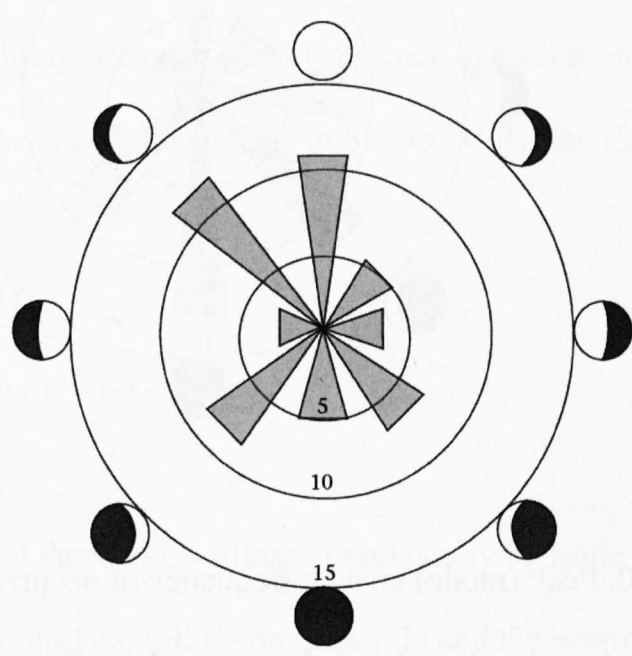


Figure 11. Median departure dates: frequency of occurrence in each moon phase for *L. vulgaris* and *L. helveticus* at Llysdimam pond, Wales, U.K. See Figure 3 for moon phases.

2.3.8 Bonferroni Correction for Multiple Testing

Of the 35 tests presented in this study, 10 showed significance, where only two would be expected by chance. Following Bonferroni correction for multiple testing (Abdi 2007), three of the data sets tested (arriving and spawning toads at Marston Pond and large amplexus events at San Ruffino) remained significant ($P < 0.001$).

2.4 Discussion

Our hypothesis that breeding phenology in amphibians reflects periodicity in the lunar cycle is strongly supported by our results, including both anurans and urodeles, from a wide range of locations across the temperate northern hemisphere. Lunar periodicity was shown for large arrival and spawning events, the number of animals in amplexus and first sightings. Only first spawning (anurans) and departure dates (*Lissotriton* and *Triturus*

newts) showed no evidence of lunar periodicity. The timing of events fell broadly into two categories: events that occurred unimodally, largely around the full moon with very few around the new moon (large arrival, amplexus and spawning events in anurans), and events that occurred bimodally such as first sighting and peak arrival events of *Lissotriton* and *Triturus* newts.

The occurrence of both unimodal and bimodal events suggests that different lunar cues are used for the two categories. Moonlight peaks once per cycle, at full moon, while gravitational pull peaks at both new and full moons (Figure 3). Large arrival, amplexus and spawning events in anurans may be triggered by moonlight, while large arrival events in newts correspond more closely to the gravitational cycle. Geomagnetism is modulated by lunar cycles (Markson 1971; Lohmann and Willows 1987), decreasing just before a full moon and peaking at the third quarter (Stolov 1965; Bell and Defouw 1966). The avoidance of the third quarter moon (phase 3) by arriving newts in unrelated studies in Wales and Sussex is striking. It is well known that amphibians, particularly newts, can detect and respond to small geomagnetic changes (Phillips 1986a, b; Sinsch 2006) and they might be using this to regulate reproductive timing.

Lunar cues could form part of the navigational system of amphibians, giving rise to lunar-related reproductive timing as a consequence. Newts are able to make use of small changes in the Earth's magnetic field for 'true' navigation (Phillips et al. 1995). However, departing newts showed no lunar periodicity, suggesting that the phenomenon is related to reproductive timing. The response of newts to the lunar cycle appears more complex than that of anurans. Statistical tests on the newt data gave variable outcomes. This could be because the tests used were not robust given the apparent bimodality of the data or because the newts are actually showing an avoidance response to the third quarter moon which Rao's test is not adequate to detect. Alternatively, the response of the newts may be complex and require more sophisticated modelling. Further work is underway to clarify this.

The random departure dates of newts suggest that, once breeding is over, other factors may have a greater influence on activity. Sinsch (1988) noticed that migrating *B. bufo* toads followed a direct path to their breeding site but a less direct one on departure. It is possible that different factors influence arrival to and departure from breeding sites in amphibians. Moonlight may act as a stimulus through either retinal or extraretinal

photoreceptors, such as the pineal gland (Takemura et al. 2006). Evidence for such mechanisms has been found in a number of species of fish, such as the rabbitfish in which cues from the moon stimulate the hypothalamus–pituitary–gonadal axis, thus controlling the secretion of gonadotrophin by the pituitary gland (Takemura et al. 2004). Where moonlight is the necessary cue for reproductive events, artificial lighting is likely to hinder synchronization among breeding populations, and may negatively affect reproductive success.

While artificial light undoubtedly affects the behaviour of amphibians (Buchanan 1993, 1998) the torchlight we used is unlikely to have biased the results of this study, as it was shone only briefly to enable animals to be counted. However, levels of ecological light pollution (ELP), which may disrupt normal amphibian breeding activity (Baker and Richardson 2006; Buchanan 2006) differed between sites. ELP is brighter and more focused than moonlight and can have a significant effect on the behaviour of amphibians which have dark-adapted eyes (Cornell and Hailman 1984).

ELP at the sites was not quantified, but all three were in Western Europe, which has high levels of light pollution (Cinzano et al. 2001). If amphibians

are responding to lunar light, ELP is likely to disrupt this response; it has been referred to as ‘the perpetual full moon’ (Longcore and Rich 2004). It may be more difficult to detect the effects of lunar phase at sites with high ELP; previous reports of lunar periodicity in amphibian reproductive activity come from areas with relatively low light pollution, such as Java (Church 1960a, b) and Western Australia (Byrne 2002).

However, observations at San Ruffino Lake suggest that anurans are not responding directly to moonlight, but may have a lunar entrained endogenous rhythm. On 2 May 2007 (a full moon) there was a large peak in toad arrivals at dusk despite cloud cover and the moon being low on the horizon behind mountains and invisible throughout the survey. It is possible that toads have an endogenous circalunar cycle, entrained at intervals by moonlight. Endogenous cycles of this type can continue for a period of time even when the Zeitgeber (entraining factor) is removed, but they need resetting periodically (Franke 1985, 1986).

Climate is likely to modify circalunar rhythms, and Fitzgerald and Bider (1974) found that lunar-related activity in *B. americanus* was masked by weather. While our data are limited to northern temperate regions, evidence

for lunar periodicity in amphibian reproduction in tropical and Mediterranean climates has been presented by Church (1960a) and Byrne (2002), respectively. In Java, where humidity and temperature vary little throughout the year, *B. melanostictus* breeds all year round; most females were in reproductive condition around full moon, regardless of temperature or rainfall (Church 1960a). Byrne (2002) observed that the number of mating pairs of the frog *C. georgiana* in Western Australia (with a Mediterranean climate of mild winters and hot summers) was correlated with lunar phase, with more matings occurring around the full moon. The apparent migration 'response' to full moon in anurans may be a consequence of oocyte development being linked to lunar phase earlier in the year, rather than a direct effect. This has been demonstrated in rabbitfish (where the lunar-entrained ovulation cycle culminates in spawning at a species-specific lunar phase (Park et al. 2006)) and in amphibians (female *B. melanostictus* mainly ovulate around the full moon; Church 1960a).

Our results described only events that occur during the amphibian breeding season. Outside the breeding season Fitzgerald and Bider (1974) reported increased movements of *B. americanus* around the new moon. Also,

Church (1960b) observed that *Rana cancrivora* come into reproductive condition during new moons. These observations are the reverse of the trend that we recorded, perhaps because different factors operate outside the breeding season, or because the nature of the lunar response is species-specific. Further investigation is needed to clarify such differences.

Breeding in full light under a full moon could be an adaptive antipredator response in *B. bufo*. Animals active in moonlight may be at an advantage if their visual acuity is better than that of their predators. For example, the hylid frog *Smilisca sila* is predated by bats, which locate frogs by their calls. On clear moonlit nights frogs could more easily see their predators and called more and from less concealed sites (Tuttle and Ryan 1982).

Visually oriented diurnal predators such as snakes and birds of prey will be at a disadvantage at low light intensities such as around the new moon (Oseen and Wassersug 2002). However, in the darker moon phases olfactory oriented predators such as foxes and rodents may have an advantage over their more visually oriented amphibian prey. Toads' predators include raptors, crows, owls, snakes, hedgehogs, otters, foxes and rodents (Beebee and Griffiths 2000). The variety of predators on anuran amphibians makes it difficult to speculate on the costs or benefits of breeding under a full moon in relation to predation. Anurans in large

breeding aggregations may also gain protection from predators by predator satiation (Ims 1990) leading to continued selection for synchronous reproduction. Synchronous breeding could also lead to synchronous metamorphosis, thought to be an antipredator strategy in *B. americanus* (DeVito 2003). Anurans could be responding to increases in the number of insect prey; however, this seems unlikely as common toads, at least, do not eat while breeding (Beebee and Griffiths 2000). Alternatively, light may be important for visual mate choice (Byrne 2002). More work is needed to clarify the adaptive function of lunar-related reproductive synchronization in amphibians. It is evident from the literature that temperature and rainfall influence reproductive timing (Hurlbert 1969; Semlitsch 1985; Reading 1998; Todd and Winne 2006). Amphibians have a temperature threshold for activity (Reading 1998), regardless of moon phase, and events occurring early in the year, such as first spawn, may be more influenced by minimum temperature than moon phase. This may explain why first spawn dates were not influenced by lunar phase in this study.

First spawn dates used in this study were all from species located at temperate latitudes 44°N to 54°N. It is likely that at other latitudes, the interaction of climatic variables with moon phase is different. Animals may

be responding to weather rather than lunar cues per se, but the case for a lunar effect on weather and how it affects amphibians is not clear and merits further investigation. Some studies report that climate varies according to the semilunar cycle (Brier and Bradley 1964; Carpenter et al. 1972), while other studies report no correlation (O'Mahoney 1965; Premachandra et al. 2005). In this study we considered only the 29.5-day synodic lunar cycle, that is, the time it takes for the Moon to return to the same phase. Other potentially important lunar cycles such as the 18.6-year nodal cycle, caused by variations in the inclination orbit of the Moon with respect to the Earth's equator, were not addressed. The nodal cycle can modulate lunar-synchronized breeding in some mammals (Kollerstrom 2004) and marine invertebrates (Dan and Kubota 1960) and might also affect amphibian migrations.

As a consequence of retrospective use of data collected for other purposes, there are some limitations to our study including inconsistencies of methods between sites, and a lack of data on light levels, moon rise and set times, or cloud cover. Data collected using pitfall traps might have given more reliable data on arrival totals. Our analysis is further limited by the lack of statistical methods to control for multiple factors when analysing circular

data. This means that where otherwise we might have pooled data sets and controlled for categorical variables such as site or species, it was instead necessary to analyse each data set separately, giving smaller sample sizes and potential issues with multiple testing.

However, we consider that multiple testing was not a serious issue in this study as significant outcomes were clustered around particular reproductive events (large anuran arrival and spawning events), whereas non-significant results were clustered around first spawn, newt departures and some newt arrivals, indicating the results have biological relevance, and are not statistical artefacts. Data were still in some cases pooled by sex; this was unavoidable where sex was not recorded (i.e. first newt arrivals, arrivals at San Ruffino in 2006, arrivals of *R. kl. hispanica*). The response of amphibians to the moon is likely to be more complex than the analysis here suggests, and further work is underway to unravel some of these more complex responses.

Despite current limitations, our results point to a clear lunar effect in amphibian breeding phenology and provide a useful starting point for further research in this area. Few studies of breeding phenology consider

lunar phase as a variable, and to our knowledge this is the first study that has investigated the effect of lunar phase on urodele reproductive timing. Population censuses and long-term monitoring should take lunar phase into account, as the number of amphibians active varies with the lunar cycle and may give inconsistent results from year to year. For example, programmes designed to avoid mass road deaths could be coordinated with the appropriate parts of the lunar cycle for a particular species. The findings of this study therefore have important implications for the conservation of amphibians, which are declining globally (Halliday 1998; Stuart et al. 2004).

Chapter 3: The effects of lunar phase on amphibians: literature review and meta-analysis

This chapter has been accepted for publication in the journal *Behavioral Ecology* under the title: Amphibians response to lunar cycles; a review of current knowledge.

3.1 Introduction

In the light of catastrophic declines in amphibian populations (Houlahan et al. 2000), population monitoring, and research into factors that influence behaviour (in particular where behaviour impacts on recruitment or mortality) are critical. While the influence of environmental variables such as temperature and rainfall on behaviour and population counts are widely recognised (Wells 2007), few studies have addressed the possible impact of lunar cycles, which might influence sampling efficiency if the numbers of animals active varies with moon phase.

Recent reports suggest that lunar cycles affect breeding and behaviour in a number of amphibian species (Deeming 2008; Grant et al. 2009). This is unsurprising as light levels can vary by nearly two orders of magnitude throughout the course of the lunar cycle (Campbell et al. 2008), providing a significant source of temporal environmental heterogeneity likely to affect nocturnal animals. Gravitational and magnetic changes also occur regularly with the lunar cycle, such that gravitational forces peak twice per cycle (at new and full moons) and the geomagnetic field peaks during the moon's 3rd quarter (Stolov 1965; Bell and Defouw 1966). Despite this, however, and although it was shown in the 1960s that ovulation in several species of tropical anurans was linked to the lunar cycle (Church 1960a, b; Church 1961), the topic has since been largely neglected.

Here, we review the literature which (either quantitatively or descriptively) describes lunar mediated behaviour in amphibians, and discuss the potential adaptive nature of such behaviour. We also make recommendations for improving comparability between studies by accounting for lunar cycles in study design, and for the correct analysis of lunar cycle data.

3.2 Evidence for lunar mediated behaviour

Literature searches revealed 79 examples of amphibian behaviour where moon phase was recorded, arising from 48 publications. These included 22 examples of amphibians showing a positive response to the full moon, by increasing activity or calling, 31 showing a negative response, 16 where amphibians were unaffected by moon phase and 11 examples that were ambiguous or related only to navigation and homing (Table 4). There were significantly more examples of amphibian behaviour being affected, rather than unaffected by lunar phase ($N=75$, $DF=1$, $X^2=20.28$, $P<0.001$). Even allowing for non-presentation of negative results, this implies that lunar-related behaviour in amphibians is considerably more prevalent than previously supposed.

Table 6. Summary of literature concerning lunar mediated responses in amphibians*

Type of response	Number of species	Species
REPRODUCTIVE ACTIVITY		
Migration or breeding linked to full moon	5	<i>Crinia georgiana</i> , [1] [2]a; <i>Bufo melanostictus</i> (ovulation) [3][4]; <i>Bufo biporcatus</i> [4]; <i>Bufo bufo</i> [5]; <i>Rana temporaria</i> [5]; <i>Pyxicephalus adspersus</i> [6]
Migration or breeding linked to new moon	1	<i>Fejervarya (Rana) cancrivora</i> [7]
Migration or breeding unaffected by moon phase	3	<i>Ambystoma californiense</i> [8]; <i>Bufo calamita</i> [9]; <i>Bufo melanostictus</i> (spermatogenesis) [3][4]
Males call more, or for longer under increased moonlight	11	<i>Ptychadena stenocephala</i> [10]; <i>Bufo Asper</i> [11][12]; <i>Cophixalus ornatus</i> [13]; <i>Smilisca sila</i> [14], [15]; <i>Acris crepitans</i> [16]; <i>Bufo biporcatus</i> [12]; <i>Megophrys montana</i> [12]; <i>Dendropsophus bipunctatus</i> [17]; <i>Hypsiboas albomarginatus</i> [17]b; <i>Leptodactylus aff. bokermani</i> [17]b; <i>Kassina senegalensis</i> [18] c
Males call more, or for longer under decreased moonlight (including full moon avoidance)	21	<i>Hyla cinerea</i> [16]; <i>Hyla versicolor</i> [19]; <i>Bufo hemiophrys</i> [19]; <i>Bufo cognatus</i> [19]; <i>Pseudacris maculata</i> [19]; <i>Rana pipiens</i> [19]; <i>Hyla crucifer</i> [20]; <i>Hyla squirrella</i> , [21]; <i>Hyla boulengeri</i> [22]; <i>H. elaeochroa</i> [22]; <i>H. ebraccata</i> [22]; <i>H. phlebodes</i> [22]; <i>H. loqax</i> [22]; <i>Smilisca baudini</i> [22]; <i>S. phaeota</i> [22]; <i>S. puma</i> [22]; <i>Phyllomedusa callidryas</i> [22]; <i>P. saltator</i> [22]; <i>Leptodactylus mystacinus</i> [17]; <i>Rhacophorus achantharrhena</i> [23]; <i>Physalaemus pustulosus</i> [24]d
Male calling is unaffected by moon	10	<i>Cophixalus ornatus</i> [25]; <i>Austrochaperina robusta</i> [25]; <i>Bufo woodhousii</i> [19]; <i>Spea bombifrons</i> [19]; <i>Hyla boans</i> [26]; <i>Rana clamitans melanota</i> [27]; <i>Dendropsophus anceps</i> [17]; <i>D. minutus</i> [17]; <i>D. seniculus</i> [17]; <i>Physalaemus signifer</i> [17]
Mate choice affected by moon phase	2	<i>Physalaemus pustulosus</i> [28]; <i>Hyperolius marmoratus</i> [29]
NON REPRODUCTIVE ACTIVITY		
Animals using moon for orientation, navigation, homing	3	<i>Triturus alpestris</i> [30]e <i>Acris gryllus</i> [31]; <i>Bufo fowleri</i> [32]
Animals not using moon for orientation, navigation, homing	2	<i>Rana pipiens</i> [33]; <i>Bufo boreas</i> [34], [35]
Foraging / Locomotion / abundance increased at new moon (including full moon avoidance)	7	<i>Lissotriton vulgaris</i> [36]; <i>Triturus cristatus</i> [36]; <i>Bufo americanus</i> [37]; <i>Hoplobatrachus occipitalis</i> [38]; <i>Leiopelma hamiltoni</i> [39]; <i>Bufo fowleri</i> [40]; <i>Ambystoma opacum</i> (larvae) [41]; <i>Hypsiboas leptolineatus</i> [42]
Foraging / Locomotion / abundance increased at full moon	2	<i>Litoria sp</i> [43]; <i>Eleutherodactylus martinicensis</i> [44] ; <i>Pyxicephalus adspersus</i> [6]
Foraging / Locomotion / abundance unaffected by moon	3	<i>Rana clamitans melanota</i> [27]; <i>Phaeognathus hubrichti</i> [45]; <i>Eleutherodactylus augusti</i> [46]
Ambiguous cases	5	<i>Lissotriton vulgaris</i> [5]; <i>Triturus cristatus</i> [5]; <i>Lissotriton helveticus</i> [5]; <i>Rana sylvatica</i> [19]; <i>Bufo houstonensis</i> [47]; <i>Bufo bufo</i> [48]

^aAnother study found the number of males in the chorus was not affected by the lunar cycle; ^bclose to significance at $P=0.06$; ^cterritorial, not mating call on a full moon; ^dwhen bat predators present; ^ecould be an artefact of the testing arena.

1.Byrne and Roberts 2004; 2.Byrne 2002; 3.Church 1960a; 4.Church 1961; 5.Grant et al 2009; 6. Yetman et al. 2011; 7.Church 1960b; 8.Loredo and Van Vuren 1996; 9.Sinsch 1988; 10.Amiet 1974; 11.Inger 1969; 12.Iskandar 1998; 13.Brooke et al 2002; 14.Da Silva Nunes 1988; 15.Tuttle and Ryan 1982 ; 16.Granda et al 2008; 17.Abrunhosa et al 2006; 18.Fleischack and Small 1978; 19.Johnson and Batie 2001; 20.Forester and Lykens 1986; 21.Taylor et al 2007; 22.Duellman 1967; 23.Harvey et al 2002; 24.Rand 1995; 25.Hauselberger and Alford 2005; 26.Magnusson et al 1999; 27.Baker and Richardson 2006; 28.Rand et al 1997; 29.Backwell and Passmore 1990; 30.Diego-Rasilla et al 2005; 31.Ferguson et al 1965; 32.Ferguson and Landreth 1966; 33.Dole 1972; 34.Gorman and Ferguson 1970; 35.Tracy and Dole 1969; 36.Deeming 2008; 37.Fitzgerald and Bider 1974; 38.Kühn et al 1987; 39.Newman 1990; 40.Ferguson 1960; 41.Hassinger and Anderson 1970; 42.Hiert and Moura 2010; 43.Brown and Shine 2002; 44.Jaeger and Hailman 1976; 46.Goldberg and Schwalbe 2004; 47.Swannack et al 2009; 48.Lluisa et al 2010.

* Literature was searched systematically in May 2010, using ISI Web of Knowledge (<http://wok.mimas.ac.uk/>), Google Scholar (www.google.com), Amphibiaweb (www.amphibiaweb.org) and JSTOR (<http://www.jstor.org/>). Search terms for WOK and Google Scholar were amphibian* and (behaviour OR behaviour, subsetting using lunar OR moon*), in Amphibiaweb “moon” or “lunar” were entered in the field “Account Text”, and in JSTOR “amphibian moon” and “amphibian lunar” were used. In Google Scholar we also searched for (All the words “amphibian, lunar, moon” and at least one of the words “reproduction, breeding, ovulation, spawning”) in order to incorporate non-behavioural reproductive studies. Where two studies observed a similar response in the same species, this was considered one example. Where studies observed differing responses or the behaviour studied differed, this was considered more than one example. When a study observed breeding and nonbreeding behaviour in the same species this was considered two examples. A further Scholar and ISI search was conducted immediately prior to submission of this paper in March 2011. Statistical tests were carried out using Minitab 15 and Oriana 3

For the four families which contained sufficient examples for analysis (Bufonidae, Ranidae, Salamandridae and Hylidae), we compared the prevalence of unambiguous responses to the lunar cycle, to of the number of times the lunar cycle was included as a variable. On average, 71% of studies reported a response in these four families; there was no significant difference between these families in the prevalence of lunar responses (Chi squared test: $N=43$, $DF=3$, $\chi^2 = 1.06$, $P= 0.79$).

3.3 The adaptive nature of lunar mediated behaviours

Animal behaviour is the result of a complex balance of costs and benefits; animals act to maximise their foraging efficiency and reproductive output, while minimising their predation risk. The adaptive nature of lunar mediated behaviour is therefore likely to vary within and between species, with location and habitat, and with prevailing environmental conditions, as species are expected to behave differently with regard to moon phase, depending on their unique ecology.

3.3.1 *Predator avoidance*

Lunar-related predator avoidance depends on a number of factors, including whether the visual acuity of predator and prey are similar. For example, visual nocturnal predators (e.g. owls) may be at an advantage under increased light levels, but if changes in illumination affect both visual predator and prey equally, there will be no net change in predation risk (Rand 1997). Non-visual nocturnal predators such as bats (which use echolocation), carnivorous mammals (which use visual and olfactory cues)

or snakes (which use olfactory and vibrational cues, as well as visual ones) may be at a disadvantage at full moon if their amphibian prey can visually detect and avoid them. For diurnal predators (e.g. corvids), full moon can provide opportunities to hunt crepuscular or nocturnal prey, due to increased light levels at dusk. Overall, the relative advantages to prey and predators under different levels of moonlight depends on the combination of senses used by prey and predator, the range of vision under which each best operates, and consequently whether a particular level of moonlight gives predator or prey a visual advantage. Each amphibian species has a range of illumination under which it can see best, and in some species this range may be very narrow, and much wider in others (Jaeger and Hailman 1976), so relative advantage is likely to be highly species-specific.

The behaviour of conspecifics and other competitors further complicates the picture (Daly et al. 1992). Many amphibians, for example, prey on insects, and are in turn preyed upon by a range of carnivores and omnivores. All three categories (insect prey, amphibian predator and top predator) will therefore be involved in a multiparty game situation, the outcome of which is difficult to predict and depends on the relative costs and benefits, which will vary with the game situation (Seligman 2007). For

this reason the emergent behaviour in relation to lunar cycles may be difficult to predict.

A number of amphibian behavioural responses to the lunar cycle have been attributed to predator avoidance, including changes in the timing or intensity of calling (Taylor et al. 2007, Granda et al. 2008), foraging and other activity outside the breeding season (Fitzgerald and Bider 1974), departure from breeding ponds (Deeming et al. 2009), and visual signalling / mate choice (Backwell and Passmore 1990; Rand et al. 1997).

Calling is important in some anurans as an advertisement for reproduction, but can make them conspicuous to predators, so the timing, duration and location of displays should be evolutionarily optimised to maximise reproductive success, while minimising energetic cost and predation risk (Brooke et al. 2000). Calling is the best documented behaviour type in relation to lunar cycles (42 of our 75 examples, Table 6), and illustrates the diversity of responses exhibited by different taxa. Many anurans confine calling to the darker parts of the lunar cycle; *Hyla cinerea*, for example, calls at considerably fewer listening points in a call survey under increased

illumination (Granda et al. 2008), while the Squirrel treefrog (*Hyla squirrel*) either stops calling on moonlit nights, or calls from concealed sites (Taylor et al. 2007). Predator avoidance has been suggested as the reason for this behaviour (Taylor et al. 2007, Granda et al. 2008). Conversely however, some species call more during full moon. *Acris crepitans*, for example calls more in moonlight (Granda et al. 2008). *A. crepitans* is a small and cryptically coloured frog, which has high frequency rapidly extinguished calls, making it difficult for both visual and auditory predators to locate, and Granda et al. (2008) speculate that it may call during periods of high moonlight to avoid acoustic interference by the calls of the other frogs, of which there are several sympatric species.

Because calling behaviour is often associated with breeding, however, it is difficult in many cases to discriminate whether the species concerned are responding to lunar cycles due to predator avoidance, or because they are using lunar cues to aid breeding synchronisation. Few studies explicitly investigate predator avoidance with respect to lunar cycles. Two studies relating calling behaviour to bat predation are exceptions to this, but reveal differing responses to lunar illumination, although both responses indicate that moonlight helps these amphibians to detect their predators (Tuttle and

Ryan 1982; Ryan 1985). The túngara frog *Physalaemus pustulosus* and the hylid treefrog *Smilisca sila* are both preyed on by bats. On clear and moonlit nights túngara frogs reduce calling when bats arrive, whereas on cloudy moonless nights there is little change in chorusing behaviour (Ryan 1985). Conversely *S. sila* call for longer, and from more open sites on moonlit nights (Da Silva Nunes 1988), and when presented with model bats, they increase the number and complexity of calls when moonlight is simulated, and decrease calling during low light conditions (Tuttle and Ryan 1982).

Other examples of lunar adapted behaviours also show a diversity of response to the lunar cycle. Activity (number of frogs encountered per night) of several frogs of the genus *Litoria* is significantly greater on illuminated nights, possibly as an adaptation to predation by snakes. Snakes are a partially olfactory predator and may well be at a relative disadvantage to their visually oriented prey under increased illumination around the full moon (Brown and Shine 2002). Activity in brighter lunar phases as an adaptation to snake predation has been observed in other taxa (Bouskila 1995), and as snakes are major amphibian predators (Wells 2007) this may be a common response. The Caribbean frog *Eleutherodactylus*

martinicensis is more active, and prefers more open habitats, on moonlit rather than moonless nights (Jaeger and Hailman 1976). Its predators are small carnivorous mammals (amphibiaweb; accessed 12.8.10) which may be less visually oriented than *E. martinicensis*, giving the frog a visual advantage in moonlight.

The American toad *Bufo americanus* shows the reverse response, and exhibits reduced locomotor activity during full moon phases outside the breeding season (Fitzgerald and Bider 1974). Similarly, captures (in bottle traps) of *Lissotriton vulgaris* and *T. cristatus* during spring were highest around the new or waning moon, respectively (Deeming 2008); because bottle traps were facing towards the pond, it is assumed that this reflects non-breeding behaviour (departures) rather than breeding arrivals. These moonlight avoidance behaviours may be an antipredator adaptation but this was not tested.

Two examples show that variation in predation risk across the lunar cycle can affect mate choice in amphibians, and therefore may affect reproductive success. Female reed frogs *Hyperolius marmoratus* prefer simulations of

males calling from elevated perches surrounded by upright vegetation rather than open ground at the full moon, but showed no such preference when ambient light levels were low (Backwell and Passmore 1990).

Female túngara frogs (*P. pustulosus*) are more willing to respond to calls from males when conditions are dark, (Rand et al. 1997), possibly because they are safer from visual predators at this time. This is in contrast to males of this species which are additionally at risk from acoustic predators (bats) and consequently call more in high illumination (Rand et al. 1997). The question of how moonlight impacts on visual display and mate choice in amphibians merits further research.

Despite an intuitive assumption that amphibians might avoid activity at full moon in order to reduce predation risk, we have found no significant difference in the number of studies showing increased, versus decreased, activity during brighter moon phases (either for all studies [20 show an increase, 30 show a decrease; Chi-squared test: $N=50$, $DF=1$, $X^2=2.0$, $P=0.16$] or for calling behaviour in isolation [11 show an increase, 21 show a decrease; Chi-squared test: $N=32$, $DF=1$, $X^2=3.1$, $P=0.08$]). This reflects

the highly species-specific nature of the costs and benefits associated with nocturnal activity under different levels of illumination.

3.2.2 *Reproductive synchronisation*

Reproductive synchronisation serves to maximise reproductive success, by maximising the pool of available breeding adults (Shapiro et al. 1993; Tsukamoto et al. 2003) and by reducing predation risk through ‘predator dilution / satiation’ (Ims 1990). Predator dilution or satiation can exert protective effects for breeding adults in the short term (Ims 1990), as well as enhancing recruitment by resulting in predator satiation later in the life cycle, through synchrony of larvae or metamorphs (Omori 1995).

Use of the lunar cycle to synchronise reproduction in amphibians was first reported in the 1960s, for *Bufo melanostictus* in Java and Indonesia (Church 1960, 1961) and *B. biporcatus* in Bali (Church 1961). In these tropical habitats temperature and humidity vary little throughout the year, food is always available, and toads breed all year round. Ovulation in *B. melanostictus* (measured by destructively sampling the ova) was associated

with the full moon (Church 1960), and amplexus was more common when the moon was waxing (Church 1961; later statistically validated by Deeming 2008), while in Bali, more *B. biporcatus* females were found in the water when the moon was waxing.

Lunar synchronised reproduction in amphibians is not again discussed in the literature until some forty years later. Various studies then report lunar phase effects, in Europe, Africa and Australia. For example, large arrival and amplexus events (common toad, *Bufo bufo*), and large spawning events (*B. bufo* and common frog, *Rana temporaria*), are more frequent around full moon at a variety of European sites (Grant et al. 2009). In the African giant bullfrog, *Pyxicephalus adspersus*, spawning is more prolonged when there is a full moon (Yetman et al. 2011). In Australia, correlations are recorded with frequency of mating and activity level (increased near full moon in the Australian quacking frog, *Crinia georgiana*, Byrne 2002), male density, operational sex ratio and number of females in a chorus (negatively correlated with days since a full moon in *C. georgiana*, Byrne and Roberts 2004). Byrne and Roberts (2004) showed that most of the variation in male density and the number of females in a chorus was explained by lunar phase and rainfall, but it should be noted that Smith et al. (2003) found that

variation in male chorus attendance of *C. georgiana* at a nearby site was explained by temperature, the number of females in the chorus and date, and there was no significant correlation with rainfall or moon phase; the reason for the difference is unclear.

Mechanisms to aid synchronisation of reproduction, such as lunar cues, may be particularly important for animals that live in dispersed populations, or those whose reproduction is highly synchronised. In amphibians, this could relate to highly terrestrial amphibians such as the common toad, which are spatially dispersed during overwintering, and to explosive breeding species such as *B. bufo* and *R. temporaria* (where males seek out females by scramble competition rather than attracting them by calling (Höglund and Robertson 1987; Ryser 1989)).

A mechanism by which lunar synchrony might be controlled is described for rabbitfish (a group of fish species in the genus *Siganus*), where lunar light inhibits the production of melatonin by the pineal gland, which in turn influences the secretion of reproductive hormones, thereby synchronising reproduction (Takemura et al. 2006). In lunar synchronised fish, spawning

may be synchronised to the full, new or both moons, and species within the same family may spawn at different species-specific lunar phases (Takemura et al. 2004). Similarly in amphibians, although most of the examples cited above show synchronisation around the full moon, there are exceptions; Church (1960b, in Java) found more *Fejervarya cancrivora* (formerly *Rana cancrivora*) with mature ova around the new moon. Hormone mediated light-entrained ovulation is therefore likely to be a proximate, but not ultimate cause of lunar synchronised reproduction.

As well as having the potential to influence reproductive cycles through physiological mechanisms, lunar phase could influence reproductive timing indirectly through effects on predation risk (as described above), or on mate choice. There are several examples of amphibians using visual signals in selecting mates (Summers et al. 1999; Rosenthal et al. 2004; Hettyey et al. 2009). Byrne (2002) suggested that the importance of light in mate choice might explain the increased frequency of mating of *Crinia georgiana* around full moon, but this was not explicitly tested. Given the variability in amphibian visual acuity at different levels of illumination (Jaeger and Hailman 1976), and the relatively common use of visual signalling, the possibility that lunar light aids mate choice in some groups is an interesting

area for future research. One interesting example suggests that moonlight could influence visual signalling in amphibians; the African frog *Kassina senegalensis* gives a territorial call (which is distinct from its mating call) only on full moon nights when males are spaced less than 1 m apart (Fleischack and Small 1978).

3.3.3 Foraging

Insects form a major component of amphibian diet (Wells 2007), and there are numerous reports of insects changing activity with the lunar cycle (although evidence is contradictory and may be at least partially due to artefacts caused by light traps) (Nowinszky 2004). It therefore seems likely that variation in amphibian activity with lunar cycles will reflect changes in prey availability. Only two examples of lunar mediated amphibian behaviour appear to be a response to prey availability, however, and both are somewhat ambiguous.

Fowler's toads (*Bufo fowleri*) in Mississippi, USA, congregate around streetlights to feed on insects; fewer toads congregate when the moon is full and the sky clear (Ferguson 1960). Research was conducted outside the

breeding season, so the movements described are unlikely to relate to reproduction. Potential predators in the area were controlled by the human population, so it seems unlikely to be a direct response to predation, although an evolved but now redundant avoidance response to predators cannot be ruled out. It seems probable that fewer toads congregate to feed on clear moonlit nights because fewer insects are attracted to streetlights when the contrast between streetlight and ambient (lunar) light is less.

The larvae of the salamander *Ambystoma opacum* rise to the surface of the water in response to rapid decreases in illumination such as that seen at a lunar eclipse (Hassinger and Anderson 1970). This may be a response to prey availability, as many species of plankton (on which larvae feed) are known to move vertically upwards in response to decreases in lunar illumination (Gliwicz 1986; Alldredge and King 2003). However, other reasons for the behaviour cannot be ruled out.

Response to the lunar cycle as a means of maximising foraging effectiveness appears to be relatively rare in amphibians, perhaps because predation and reproduction are much stronger selection pressures.

Alternatively this may be an understudied aspect of amphibian foraging

behaviour, either because it is difficult to separate the maximisation of foraging effectiveness from antipredator behaviour, or because amphibians are generally studied during their breeding season when they are more conspicuous and the terrestrial stages of life are less well studied.

3.3.4 Navigation, orientation and homing

Lunar cues influence navigation, orientation and homing in some amphibian species, such as *Acris gryllus* (Ferguson et al. 1965), *Bufo fowleri* (Ferguson and Landreth 1966) and *Triturus alpestris* (Diego-Rasilla et al. 2005), while others such as *Rana pipiens* (Dole 1976) and *Bufo boreas* (Tracey and Dole 1969, Gorman and Ferguson 1970) move randomly with respect to lunar cues. Lunar influence on navigation may be related to cyclic changes in the Earth's magnetic field, which fluctuates according to the synodic cycle, peaking at the third quarter (Stolov 1965; Bell and Defouw 1966). Some amphibian species use magnetoreception in navigation, orientation and homing, detecting very small shifts in magnetic intensity. Examples include the Eastern red-spotted newt *Notophthalmus viridescens* (Phillips et al. 1995, 2002; Phillips 1986a,b; Fischer et al. 2001; Deutschlander et al. 1999b); the Alpine newt *Triturus alpestris* (Diego-

Rasilla et al. 2005b), bullfrog tadpoles *Rana catesbeiana* (Freak and Phillips 2005) and larval Iberian green frogs *Pelophylax perezi* (Diego-Rasilla and Phillips 2007). Detection of lunar modulation of geomagnetism is suggested by the avoidance of the third quarter by newts *Lissotriton vulgaris*, *L. helveticus* and *Triturus cristatus*, when arriving at two separate breeding sites in Wales and Hampshire, UK (Grant et al. 2009) (although orientation behaviour was not investigated as part of this study). The mechanism of magnetoreception has been shown to be a light-dependent process, as both urodeles and anuran tadpoles shift their response to magnetic fields by 90° in long-wavelength light (Phillips and Borland, 1992a; Deutschlander et al. 1999a, b). Although light-dependent magnetoreception is not completely understood, this suggests a potential interaction between lunar light, magnetoreception, and amphibian orientation behaviour.

3.4 Recommendations

Synthesising and comparing the results of different studies on behavioural responses to the lunar cycle is difficult, as different investigators use different methods, and study different behavioural traits. Most studies were

designed for other purposes and mention the effect of lunar phase as a minor point, or they are natural history accounts of species, which are anecdotal rather than quantitative. In order to achieve a better understanding of lunar-related behaviours, a more consistent and quantitative approach is required.

3.4.1 *Recording data*

Animals may react directly to ambient light levels, or alternatively, changing light levels may regulate an endogenous rhythm (Neumann 1981). When endogenous periodic rhythms are established in a species, they continue even when the animal is isolated from its natural environment or held in constant conditions. Usually, these rhythms are entrained at intervals by an external factor, termed a 'Zeitgeber' (Anders 1982), which in the case of many amphibians could be moonlight. To distinguish between endogenous rhythms and direct responses it is necessary to study the animals during a lunar eclipse and note changes in behaviour (Donati 2001), or to keep the animals under constant conditions and see if the rhythm persists (Rahman et al. 2000). There are examples of both types of

response in the literature (e.g. Wikelski and Hau 1995; Donati 2001), but in most cases this difference is not discussed.

Of the quantitative studies available, some consider moon phase or number of days since a full moon as independent variables, regardless of whether the moon had risen or was obscured by cloud (Deeming 2008; Byrne 2002; Byrne and Roberts 2004; Grant et al. 2009); others use an index based on the probable light levels from the moon, by, for example, considering the period as moonless, or dark if the moon has not yet risen, or cloud cover was 100% (Brown and Shine 2002; Johnson and Batie 2001; Granda et al. 2008). While both are valid approaches, the effects they are able to detect differ. Using an index of probable light levels will not pick up endogenous lunar rhythms, as these will continue on days when the moon is obscured by cloud or has not yet risen (Grant et al. 2009). Considering only lunar phase without regard for light levels will miss effects that are directly due to variations in light intensity. We recommend that field studies of amphibians record the phase of the moon, which can also be done retrospectively using data from the United States Naval Observatory Astronomical Applications service (<http://www.usno.navy.mil/USNO/astronomical-applications>).

Ideally, both light levels and lunar phase should be recorded in field studies, as well as cloud cover, moon rise and set times and the moon's visibility.

3.4.2 *Analysing data*

Quantifying lunar phase is done in a variety of ways, including percentage of the moon illuminated, days to the nearest full moon, or days since full moon. This can lead to subtle differences in interpretation, and in some cases to serious statistical flaws.

Values recording the percentage (or fraction) of the moon illuminated (PMI values) reflect the proportion of the moon's surface which is visible and thus reflecting light from the Sun. This value is increasingly being used in the ecological literature (e.g. Fernandez-Duque 2004; Orrock et al. 2003; Lang et al. 2006). Because the moon passes more quickly through intermediate phases and spends longer in the new and full phases, there is a serious statistical bias associated with using PMI. Using data from the US Naval Oceanography Portal

(<http://www.usno.navy.mil/USNO/astronomical-applications/data-services/frac-moon-ill>) for 30/1/10 to 28/2/10 we plotted PMI on each theoretical day of a lunar month (Figure 12).

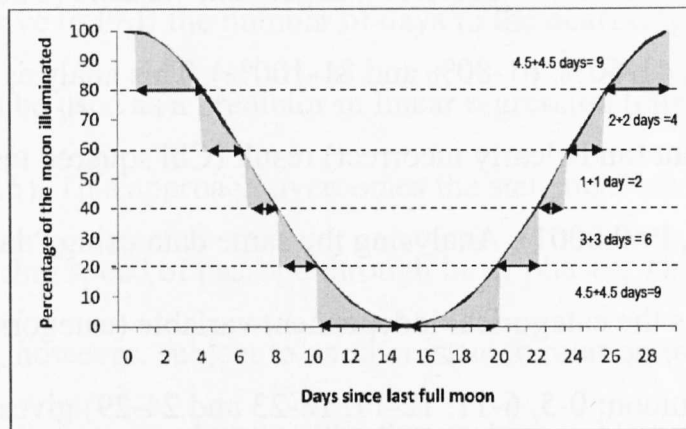


Figure 12. Shows the non-linearity of the lunar cycle with respect to days since a full moon.

It can be clearly seen that in a lunar month there are more days when the moon is 0-20% and 80-100% illuminated (nine days in each group) than days when the moon is in intermediate phases (six days in the 21-40% group, two days in the 41 to 60% group and four days in the 61-80% group. If one is counting (for example) breeding events occurring in relation to PMI, breeding is less likely in intermediate phases because there are fewer days available for breeding. To illustrate this we constructed a theoretical

model of amphibian breeding events, assuming an equal distribution of one breeding event per day, on each day of the lunar cycle, and tested for a difference between the numbers of breeding events in five equal categories using PMI as the categorical independent variable (Categories were 0-20%, 21-40%, 41-60%, 61-80% and 81-100%). This analysis produces a highly significant (and clearly incorrect) result (Chi squared test: $N=30$, $DF=4$, $X^2=63.3$, $P<0.0001$). Analysing the same data using “days since last full moon” as the categorical independent variable (categories are “days since last full moon: 0-5, 6-11, 12-17, 18-23 and 24-29) gives a nonsignificant outcome with both Chi-squared ($N=30$, $DF=4$, $X^2=0$, $P=1.0$) and the Rayleigh test (a circular goodness of fit test): ($N=30$, $Z=0$, $P=1.0$). The results obtained by Swannack et al. (2009) which showed that the endangered Houston toad, *Bufo houstonensis* does not breed when the moon is 51–60% (males) or 71–80% (females) illuminated, gives the impression that breeding does not occur in intermediate lunar phases: this conclusion may be incorrect due to uncorrected bias, although the paper does not discuss this explicitly. It is recommended that in moon phase studies, if PMI is to be used, a correction is applied to account for the fact that the moon does not spend equal numbers of days in full, new and intermediate stages. Lunar light, being directly proportional to PMI on

clear night will be subject to the same bias and this should be taken into account.

As an alternative to PMI the number of days to the nearest (past or future) full moon can be used as a predictor in linear regression (days are therefore numbered 0-15). This approach overcomes the statistical bias associated with the differing speed of passage through lunar phases. This measure and PMI are both, however, subject to another issue in relation to interpretation. There is no differentiation between the first and second halves of the lunar cycle, so the first and last quarter moons will both have the same value (approximately 50% illuminated (day 7). Equally, the days just before and after a full moon will both have values of 90-99% (days 0, 1, 2) etc. Direct effects relating to the symmetric cycles of lunar illumination would therefore be adequately described, but effects due to lunar-mediated variations in asymmetric cycles such as geomagnetism would not. Additionally, events where a lunar stage (e.g. full moon) acts as a trigger for an event which occurs subsequently (and not at the equally illuminated but preceding stage of the cycle) will also be obscured.

Finally, “days since last full moon” can be used as an independent variable (0-29), effectively distinguishing the waning and waxing phases of the moon, and effectively giving two, different values, for the same level of illumination. In this case, circular statistics should be used. Linear regression is often applied to this measure (e.g. Deeming 2008), but because moon phase is a circular variable with an arbitrary or undefined origin, linear regression will incorrectly treat day zero and 29 of the lunar cycle as outliers, not taking into account the wrapping of the scale (Hussin 2007). We therefore recommend that circular-linear regression be used for moon phase studies in amphibians, using days since last full moon as an independent variable, which can be combined with other linear variables in a multiple regression approach, as demonstrated in deBruyn and Meeuwig (2001). Alternatively, if using a categorical independent variable, circular goodness of fit tests (Batschelet 1981) can be applied (e.g. Grant et al. 2009).

3.5 Summary

Changes in amphibian behaviour in relation to the lunar cycle are widespread, both geographically and taxonomically. The way in which amphibians respond to lunar cycles cannot be generalised by taxonomic group or region, but instead appears to relate directly to species-specific ecological traits. We therefore conclude that lunar-related behaviour in amphibians is a species-specific adaptive response, primarily for the purposes of maximisation of reproductive success and predator avoidance, the final outcome of which is likely to be a trade-off between sometimes conflicting selection pressures. Lunar cues may also be used by some species to maximise foraging effectiveness, orientation / homing towards the breeding site, navigation, visual signalling and mate choice; these may prove interesting areas for further study. In particular, the gravitational and magnetic changes occurring in relation to the lunar cycle and their effects on amphibians have not been well studied. Large scale studies on the effects of moon phase on amphibians across different taxa, latitudes and mating systems are lacking and we encourage further research in this area. The results of this study have implications for the monitoring and

conservation of amphibians. Many long-term studies of amphibian diversity and population dynamics do not consider lunar phase or even record it as a variable. As the phase of the moon may have a considerable affect on the number of active or calling amphibians, long-term monitoring studies carried out on the same calendar date each year but at differing lunar phases may give misleading results. Amphibians in equatorial areas, which are particularly in need of conservation, may be especially affected by the moon.

Where responses to the lunar cycle are driven by light levels, high levels of ecological light pollution (Longcore and Rich 2004) may disrupt normal behaviour, by negatively affecting synchronisation of breeding populations, making animals more vulnerable to predation or interfering with mate choice and visual signalling. This type of disruption is more likely in areas of high light pollution such as the USA and Western Europe. Including lunar phase as a variable when attempting predictive models of amphibian breeding or activity may produce better-fitting, more accurate models. Knowledge of how the lunar cycle affects different species in different regions of the world can aid in future conservation choices.

Chapter 4: The effect of a large seismic event on the breeding behaviour of the common toad

This chapter has also been published as Grant R.A. and Halliday T.R. (2010) Predicting the unpredictable; evidence for seismic anticipatory behaviour in the common toad. *Journal of Zoology* 281:263–271.

4.1 Introduction

Research into the ability of animals to predict large seismic events such as earthquakes (EQs) has been hampered by the rarity and unpredictability of such events. EQs, unlike other natural hazards such as hurricanes and volcanoes, occur without any reliable preceding phenomena (Turcotte 1991). This precludes the design of experiments to test hypotheses concerning unusual animal behaviour in relation to large seismic events, and most such observations were recollected once the EQ had already occurred (Rikitake 1981). The inherent variability in the behaviour of animals, and the fact that much of the unusual behaviour seen in animals

before EQs is also seen in other contexts, also confuses the overall picture (Buskirk et al. 1981). Most animals observed in previous reports have been domestic animals such as dogs, chickens, cows etc. as they are normally in close proximity to human settlements (Buskirk et al. 1981). There have been fewer reports of wild animals in their natural habitat showing seismic predictive behaviour.

Much unusual behaviour shown by animals occurs shortly before an EQ, often coinciding with P-waves, which arrive a few seconds before the damaging S-waves that can be felt by humans (Buskirk et al. 1981). This response to P-waves cannot be termed a predictive response, but rather an 'early warning system' (Kirschvink 2000). Behaviour occurring several days or weeks in advance of the EQ is rarer. Fish, rodents, wolves and snakes reportedly exhibited strange behaviour up to 2 months before the Tangshan, China EQ (28/7/76, $M=7.8$) and a month before the Haicheng, China event (4/2/75, $M=7.3$), but most unusual behaviour occurred within a day or two of the event (Buskirk et al 1981). Out of 36 EQs occurring between 1923 and 1978 in Europe, Asia and the Americas, most unusual animal behaviour occurred near the epicentre within 1 or 2 days of the EQ and the species primarily reported were domestic. Fish, rodents and snakes

were the only animals that showed unusual behaviour more than a week before the event, or at some distance (450 km) from the epicentre (Buskirk et al. 1981). There are several possible mechanisms by which the prediction of seismic events by animals may occur. Animals may be able to detect seismic P-waves (which travel faster through the Earth's crust than the subsequent damaging S-waves), EQ lights (anomalous aerial luminosity) or ground tilt, all of which occur seconds to minutes before EQs (Kirschvink 2000). Groundwater anomalies, increases in humidity and changes in electrical activity may also be detected (Kirschvink 2000). Geomagnetic anomalies may also be a possible cause, particularly in animals that already have a well-developed magnetoreception system for circadian or navigational purposes (Kirschvink 2000). Alternatively animals could be detecting raised radon gas levels; there have been many reports of radon anomalies in groundwater before EQs, although they do not occur in 100% of cases (Hauksson 1981; Steinitz et al. 2003; Walia et al. 2005).

In recent years perturbations in the ionosphere have been linked with large EQs. Very low frequency (VLF) and low frequency (LF) electromagnetic signals can be used for detecting ionospheric perturbations caused by seismicity. Data from a LF transmitter in Japan showed statistically

significant correlations between EQs with a magnitude of more than 5.5, and ionospheric perturbations identified using subionospheric VLF/LF propagation (Rozhnoi et al. 2004). Superimposed epoch analysis has established that the ionosphere is disturbed a few days to a week before EQs (M46) (Maekawa et al. 2006). Also it has been shown that shallow EQs disturb the ionosphere to a much greater extent than ones that are deeper (430 km) (Kasahara et al. 2008). In this study, we recorded the activity of breeding amphibians before, during and after a strong shallow EQ. We compare this with perturbations in the ionosphere noticed before the EQ, detected by VLF sounding.

4.2 Methods

The common toad *Bufo bufo* is a mainly terrestrial amphibian, which comes to water annually to breed, stimulated by rising temperatures. Breeding occurs over a short period and large male-biased aggregations of breeding toads remain at the site until spawning is completed. In an ongoing study at San Ruffino Lake, Central Italy (43.001N, 13.384E) data were collected on the numbers of toads breeding at a shallow pool on the lakebed. A 2.5km transect was surveyed each evening at dusk from 27/3/09 to 24/4/09 and

numbers of male, female and amplexed (paired) toads were recorded. The breeding pool was inspected daily for the presence of toad spawn.

4.2.1 *Seismic data*

The EQ occurred on 6/4/09 at 01:32:39 GMT (03:32:39 local time), at L'Aquila, Abruzzo, Italy (42.334N, 13.333E), M=6.3 (Figure 13).

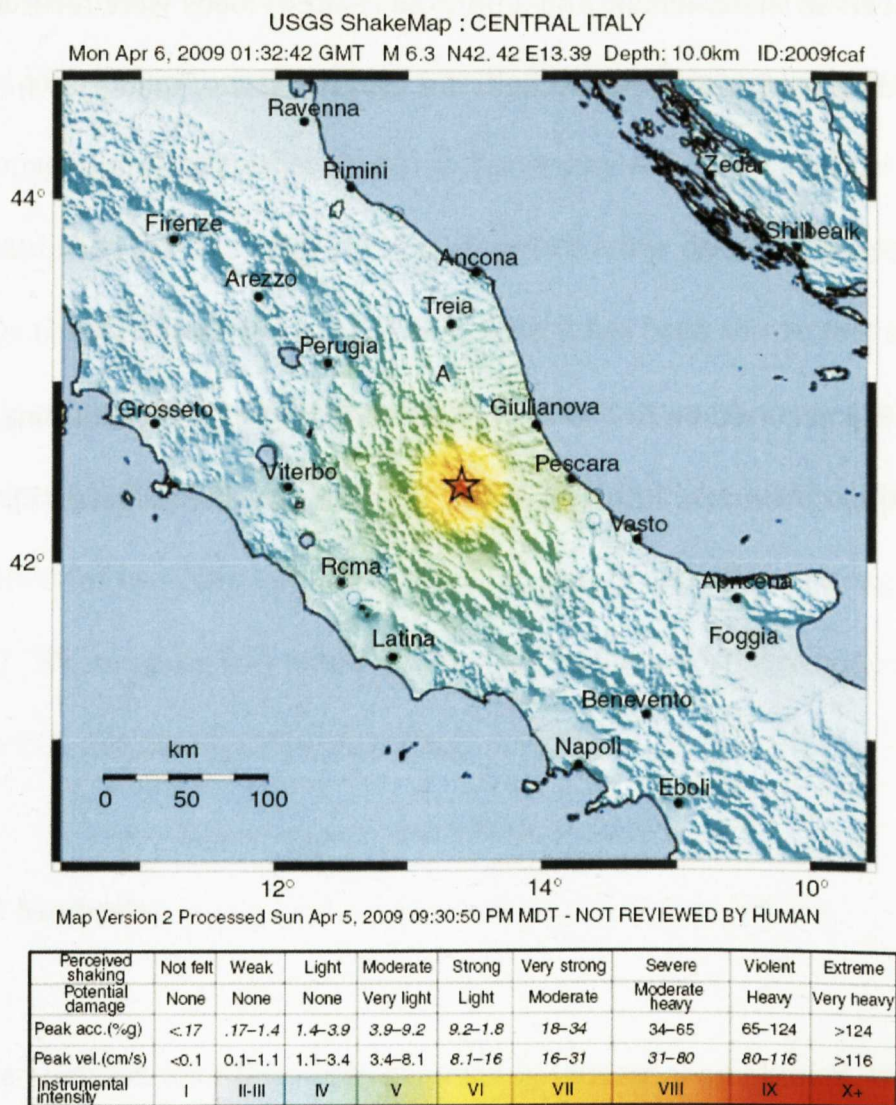


Figure 13. Showing the epicentre of the L'Aquila earthquake. From US Geological Survey. The letter A shows the approximate location of the breeding site.

The EQ was very shallow; at a depth of only 8.8 km. San Ruffino Lake is 74.29 km from the epicentre. Several aftershocks occurred that were of

sufficient magnitude to be felt in the San Ruffino Lake area (Table 7).

Seismic data were obtained from the Istituto Nazionale di Geofisica e

Vulcanologia: <http://www.ingv.it> and the US Geological Survey:

<http://www.usgs.gov>. For the purposes of this study, we considered only

aftershocks of $M > 4.5$, which is almost two orders of magnitude smaller

than the main event. In relation to toads, however, this is an arbitrary cut-

off, as we have no way of knowing the magnitude to which toads are

sensitive. The period from the main EQ to the last significant aftershock

($M > 4.5$) was designated the 'EQ period' (EP). This period was from 5/4/09

to 13/4/09. A foreshock of magnitude 4.3 also occurred, on 30/3/09 at 13:38

h (GMT) or 15:38 h local time.

Table 7 Seismic data. The dates and magnitude of the main seismic event at L'Aquila and the major ($M > 4.5$) aftershocks in the same region. Data from INGV and USGS

Date	Time (GMT)	Time (local)	Latitude	Longitude	Depth (km)	Magnitude	Area
6/4/09	01:32:39	03:32:39	42.334	13.334	8.8	6.3	Aquilano
6/4/09	02:37:04	04:37:04	42.366	13.34	10.1	4.9	Aquilano
6/4/09	23:15:37	01:15:37	42.451	13.364	8.6	4.9	Gran Sasso
7/4/09	09:26:28	11:26:28	42.342	13.388	10.2	4.8	Aquilano
7/4/09	17:47:37	19:47:37	42.275	13.464	15.1	5.5	Valle dell'Aterno
7/4/09	21:34:29	23:34:29	42.376	13.38	7	4.6	Aquilano
9/4/09	00:52:59	02:52:59	42.484	13.343	15.4	5.3	Gran Sasso
9/4/09	19:38:16	21:38:16	42.501	13.356	17.2	5.2	Gran Sasso
13/4/09	21:14:24	23:14:24	42.504	13.363	7.5	4.8	Gran Sasso

The dates and magnitude of the main seismic event at L'Aquila and the major ($M > 4.5$) aftershocks in the same region. Data from INGV and USGS. The main event is shown in bold type.

4.2.2 *Weather data*

Anecdotal or retrospective reports of unusual behaviour before EQs do not generally take into account other factors that may have affected animals, such as weather. Toad activity is strongly affected by weather, so climatic data were obtained from a weather station at Servigliano, c.15 km from the breeding site. The weather variables used in the analysis were maximum (TMAX) and minimum (TMIN) temperatures, percentage humidity (UMID), wind speed (VEL MED) and rainfall (PREC) (Table 8).

4.2.3 *Statistical analysis*

The number of days before and after the EQ and the number of days before and after the EP and the five weather variables were used as predictors in a multiple regression analysis. Day 0 was defined as 5/4/09. The number (log transformed) of male toads or amplexed pairs were the response variables. Female toads were not included due to the small sample size. Residuals were inspected to ensure normal distribution and data were checked for multicollinearity. Data were analysed using MINITAB 15.

4.2.4 VLF signal data

Subionospheric VLF/LF propagation is a widely used technique for studying ionospheric perturbations, such as those caused by seismic activity and a detailed description of the methods used for the calculation of VLF signal anomalies is described in detail in several papers (e.g. Rozhnoi et al., 2005; Maekawa et al., 2006). However a brief summary is given here.

Because daytime signals are subject to large variations, night time signals are used. To reduce the effect of seasonal and monthly variation, residual phase and amplitude values are used (dP and dA, respectively). The residuals are defined as the difference between these values and the values obtained by averaging several ‘quiet’ days. A quiet day is defined as one in which there are no phenomena likely to disturb the ionosphere such as magnetic storms, cyclones, etc. (Rozhnoi et al., 2004). Where (A) and (P) are the averages obtained for the quiet periods, and A and P are the mean phase and amplitude of the current day, then:

$$dA=A-(A)$$

$$dP=P-(P)$$

The raw radio sounding data for the L’Aquila EQ were not available, however, graphs showing the paths of the VLF signals and the

perturbations observed were obtained from a recent publication (Rozhnoi et al. 2009). These show residual phase and amplitude data obtained from reception stations at Moscow (MOS), Graz and Bari (~3000, ~1000 and ~500 km from the EQ epicentre, respectively) receiving VLF signals from a transmitter in Sardinia (ICV – 20.27kHz) and a transmitter in Sicily (ITC/NSY – 45.9kHz) whose paths crossed the seismic area (Figure 14) (Rozhnoi et al. 2009). As a control, VLF signals from transmitters in Iceland (NRK–37.5 kHz), Great Britain (GBS/GBZ – 19.58 kHz) and Germany (DHO – 23.4 kHz) were used as the path of these transmissions does not pass near the epicentre (Figure 14). In this paper, we present the data from two paths that pass near the epicentre (MOS-ICV and MOS-ITS) along with two control paths (GBS-MOS and NRK-MOS) (Rozhnoi et al. 2009).

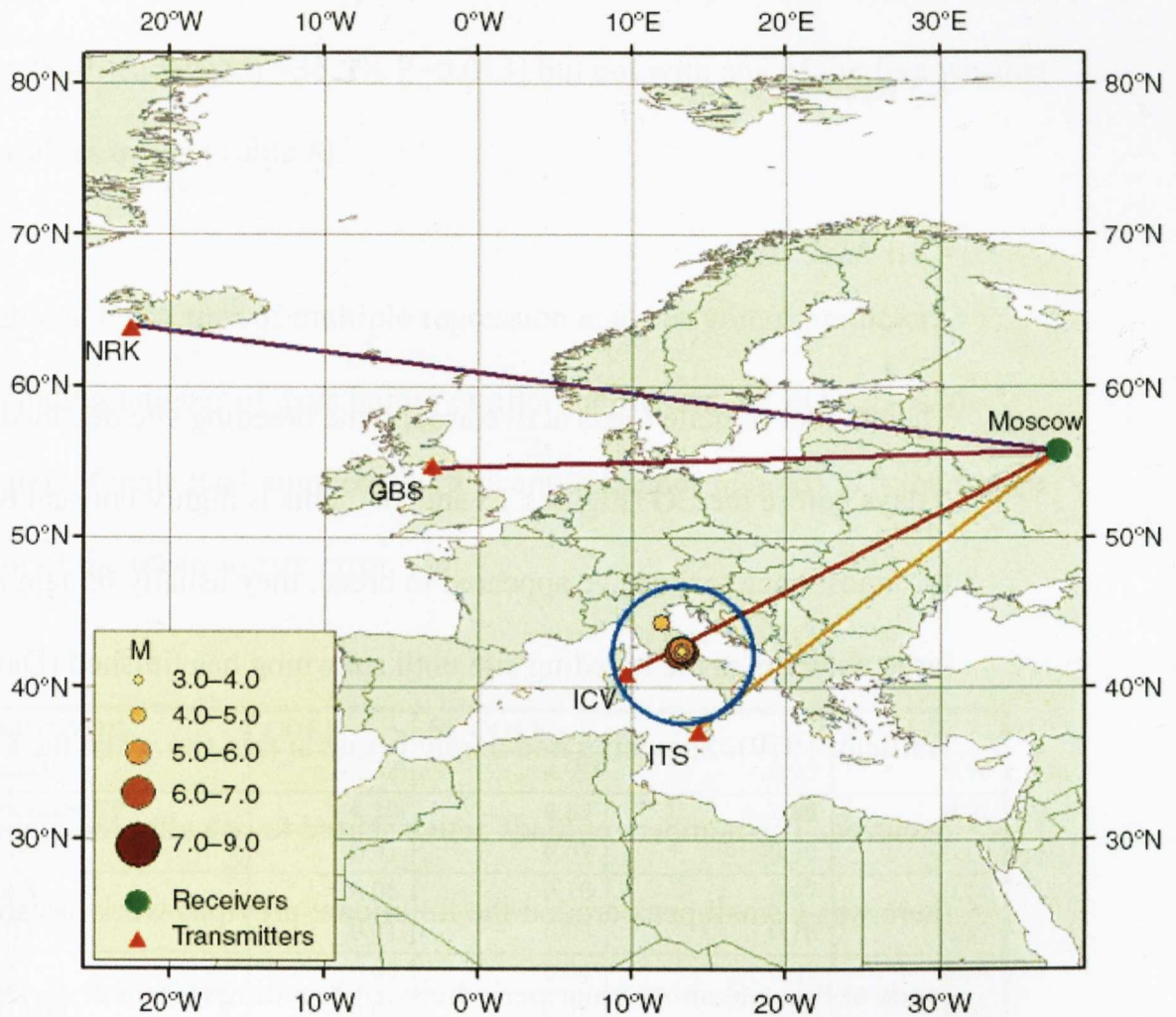


Figure 14. Showing the epicentre of the L'Aquila earthquake, the very low frequency propagation paths and the location of four transmitters and the receiver in Moscow. Moscow ICV and Moscow ITS pass through the ground projection (blue circle) of the perturbed zone. Moscow NRK and Moscow GBS are control paths as they do not pass near the epicentre. From Rozhnoi et al. (2009).

4.3 Results

4.3.1 *Male toads*

The number of male toads active around the breeding site declined by 96% 5 days before the EQ (Figures 15 and 16). This is highly unusual behaviour for toads; once toads have appeared to breed, they usually remain active in large numbers at the breeding site until spawning has finished (Davis and Halliday 1979). Spawning had barely begun at this site when the EQ occurred. The numbers of toads active started to rise after the main EQ and there was a small peak around the full moon; previous work has shown that toads at this site show lunar periodicity of breeding (Grant et al. 2009) (Figs. 3 and 4). However, numbers arriving around the full moon were reduced compared to previous years, as only 34 toads were seen, whereas the number seen in previous years ranged between 67 and 175. Numbers of active toads remained lower than usual until 15/4/09, 10 days after the EQ and two days after the last major ($M=4.5$) aftershock. Multiple regression analysis showed that the number of male toads active at the breeding site was related to the number of days before or after the EQ: [$n=28$; R^2

(adjusted) =34.8%, P=0.017] and the number of days before or after the EP [n=28, R² (adjusted) =36.5% P=0.013] but not with any of the five weather variables tested (Table 8).

Table 8. Outcomes of multiple regression analysis with 6 predictors, including number of days before or after the EP and the EQ versus the logten of male toad numbers (significant outcomes in bold). S = the square root of the mean square error.

PREDICTOR	COEF	SE COEF	t	P
Constant	-0.22	4.05	-0.05	0.96
Days since EP	0.10	0.03	2.88	0.01
TMIN	-0.01	0.07	-0.20	0.84
TMAX	0.06	0.10	0.67	0.51
UMID	-0.01	0.03	-0.16	0.87
PREC	0.03	0.04	0.97	0.34
VEL MED	0.02	0.26	0.09	0.93
	S = 0.51	R-Sq = 50.6%	RSq(adj) =36.5%	p=0.013
Predictor	Coef	SE Coef	t	P
Constant	1.87	3.70	0.51	0.62
Days since EQ	0.07	0.02	2.74	0.01
TMIN	0.01	0.06	0.17	0.87
TMAX	0.01	0.09	0.06	0.95
UMID	-0.02	0.03	-0.70	0.49
PREC	0.03	0.04	0.97	0.34
VEL MED	0.15	0.25	0.59	0.56
	S=0.52	R-Sq=49.3%,	RSq(adj)=34.8%,	p=0.017

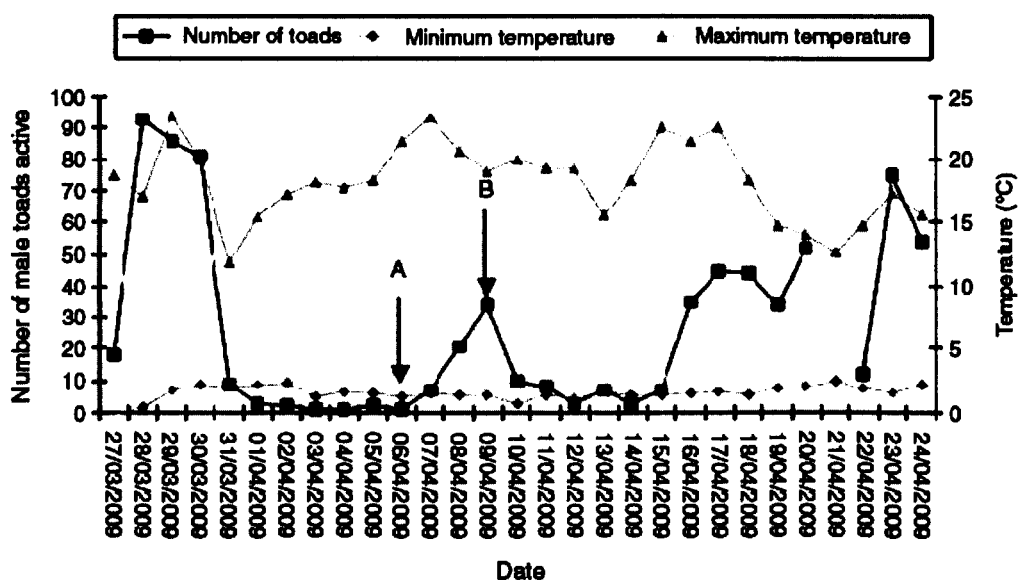


Figure 15. Number of male toads (*Bufo bufo*) active at the breeding site with maximum and minimum temperatures. A indicates the date of the earthquake, B is the date of the full moon.

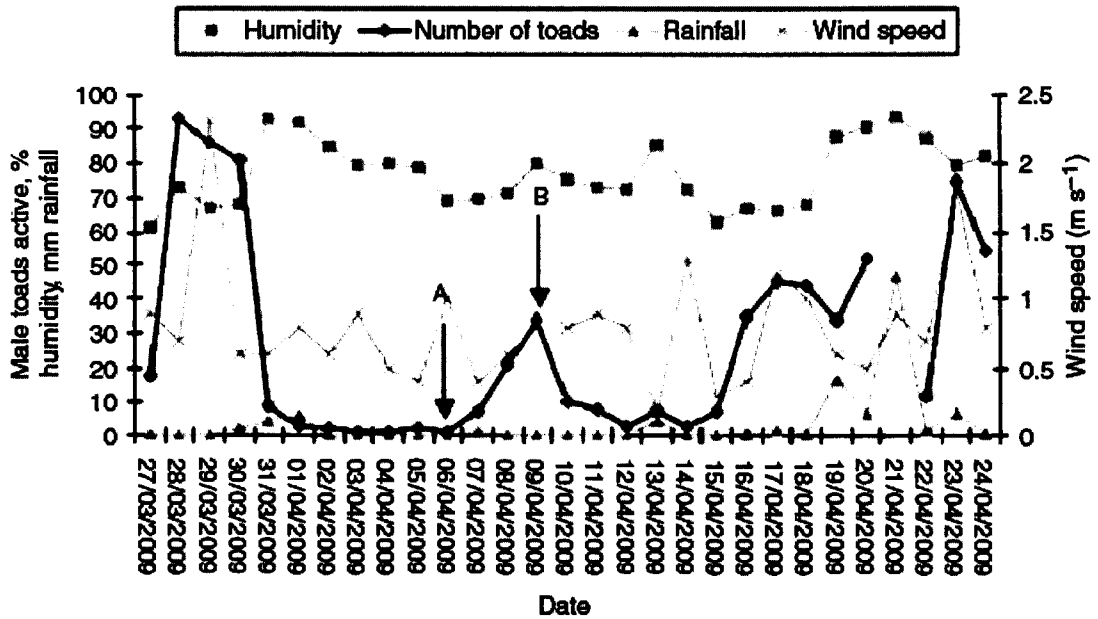


Figure 16. Number of male toads (*Bufo bufo*) active at the breeding site and humidity and rainfall. A indicates the date of the earthquake, B is the date of the full moon.

4.3.2 Amplexed pairs

The number of amplexed pairs seen declined to zero 3 days before the EQ and stayed low during the EP (Figure 17). The number of amplexed pairs was related to the number of days before or after the EQ [$n=28$, R^2 (adjusted) =13.6%, $P=0.031$] and the number of days before or after the EP [$n=28$, R^2 (adjusted) =24.6% $P=0.004$]. Fresh spawn was observed in the

breeding pond on 30/3/09, 6 days before the main EQ and on 20/4/09, 6 days after the EP. No fresh spawn was observed during the EP.

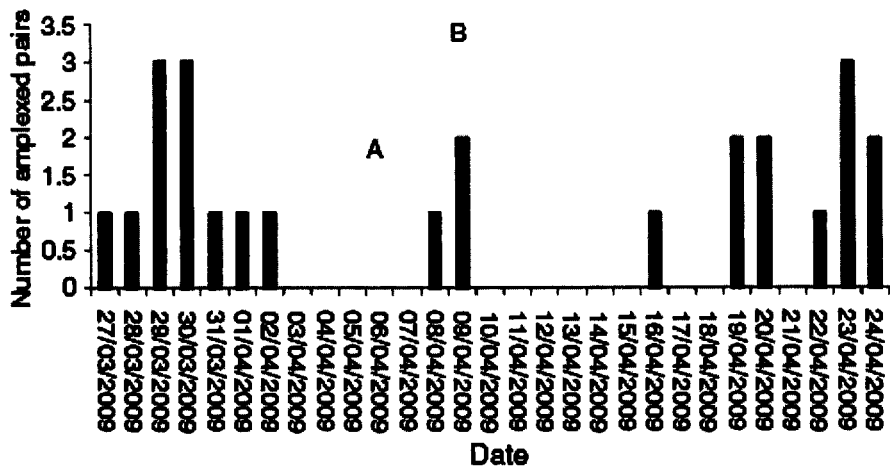


Figure 17. The number of mating pairs of toads *Bufo bufo* observed each day during the breeding season of 2009. A: The arrow shows the date of the 6.3 magnitude earthquake at L’Aquila. B: The arrow shows the date of the full moon.

4.3.3 VLF signals

The VLF signal data clearly show perturbations in the ionosphere 5–0 days before the EQ in both paths that passed near the epicentre, but not in the

control paths (Figures 18 and 19). These pre-seismic perturbations coincide with the period during which toads were no longer seen the breeding site.

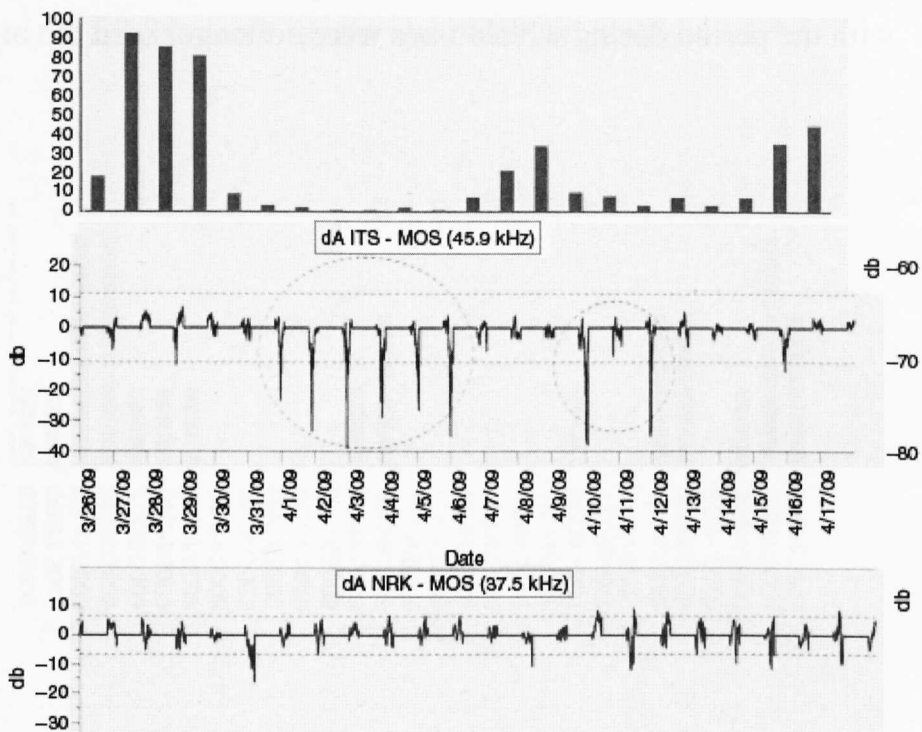


Figure 18. Numbers of male toads *Bufo bufo* active at the breeding site (above) and ionospheric perturbations sounded by very low frequency (VLF) radiowave propagation for the Moscow – ITS path which passed near the epicentre and one of the control paths (Moscow–NRK). The x-axis shows number of male toads (above) and the amplitude of disturbance in the VLF signal (decibels). The y-axis shows date. From Rozhnoi et al. 2009.

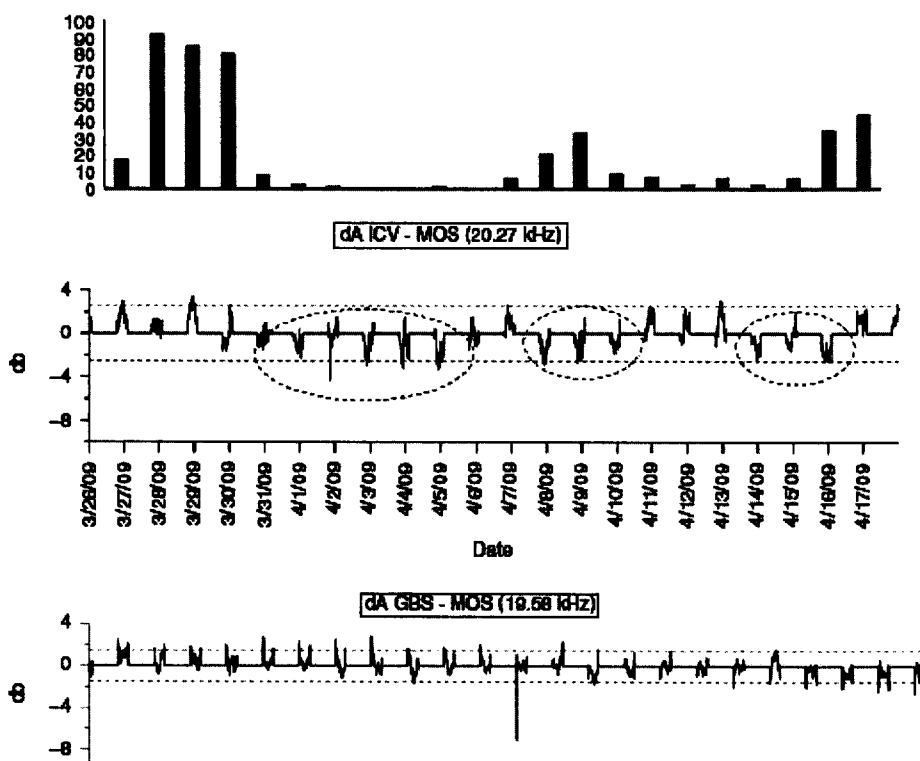


Figure 19. Numbers of male toads *Bufo bufo* active at the breeding site (above) and ionospheric perturbations sounded by very low frequency (VLF) radiowave propagation for the Moscow – ICV path which passed near the epicentre and one of the control paths (Moscow-GBS). The x-axis shows number of male toads (above) and the amplitude of disturbances in the VLF signal (decibels). The y-axis shows date. From Rozhnoi et al. (2009).

4.4 Discussion

There are few scientific studies observing animals before, during and after an EQ and the limited evidence available is contradictory. Lighton and Duncan (2005) were able to observe the behaviour of ants *Messor pergandei* during the Landers EQ, Mojave Desert, California (28/6/92; $M=7.4$). Various physiological and behavioural parameters were studied such as trail traffic rates to and from the colony, trail speed, worker mass distributions and rates of aerobic catabolism. The EQ had no effect on any of these measured variables. Yosef (1997) observed a variety of avian species before, during and after a strong EQ in Eilat, Israel (22/11/95; $M=7.2$ at 06.16 h local time). Unusual behaviour was observed by several flocks of birds at 06:00 h including several hundred gulls (*Larus* spp.) and several other species (e.g. the pied kingfisher *Ceryle rudis* European kingfisher *Alcedo atthis* and the cormorant *Phalacrocorax carbo*) which left the area and flew north. The size and direction of travel of the flock of gulls was unusual for the time of year. Also a flock of grey herons *Ardea cinerea*, took to the air at 06:08 h in an easterly direction towards Jordan. However, other small avian species (chiffchaffs *Phylloscopus collybita*, bluethroats *Luscinia svecica*, redstarts *Phoenicurus phoenicurus*, Spanish

sparrows *Passer hispaniolensis* and little green bee-eaters *Merops orientalis*) did not take flight until the first foreshock at 06:15 h and they hovered above the bushes until 06.25 when the main shock was over. Yosef (1997) continued to study the flock of herons on 10 subsequent days through 37 tremors. It was found that herons only responded (by showing signs of restlessness or by taking flight) to tremors larger than $M=4.3$. Birds' reactions to the tremors consistently occurred 30–60 s before their detection by humans. This suggests that herons' responses to smaller tremors are due to detection of P-waves or other stimuli that occur close to the event, rather than an anticipatory response. However gulls' and herons' response to the main event occurred 20 and 12 min before the shock, and may have been a genuine anticipatory response. Snarr (2005) observed the behaviour of primates (mantled howlers *Alouatta palliata*) to a seismic event occurring on 13 February 2001 ($M=6.6$; 08:22 h local time) at a distance of 341 km from the epicentre. The primates showed no unusual pre-seismic behaviour, becoming disturbed only once the EQ had occurred. However, most previous reports of animal behaviour before EQs occurred within 100 km of the epicentre (Buskirk et al., 1981) so it is possible that the study site was too far away from the epicentre to induce preseismic behavioural change, should this exist. The circadian rhythms of mice were

disrupted 3 days before the Sichuan province, China EQ (12/5/2008, M=8) and locomotor activity decreased significantly on day 3 (Li et al. 2009). The circadian rhythms of mice were also disrupted 1 day before the Kobe, Japan (17/1/95, M=7.3) EQ but in this case locomotor activity was increased to several times the usual level (Yokoi et al. 2003).

The results obtained in this study support the hypothesis that the common toad *B. bufo* is able to predict large seismic events and adjust its behaviour accordingly. Evasive action taken before EQs is likely to reduce mortality in populations (Kirschvink 2000). The absence of toads from the site before the EQ suggests they may have left the site, possibly to find higher ground which would be less at risk from rock falls, landslides and flooding.

Animals may change their reproductive behaviour when they are aware of an impending EQ or other natural disaster, for example by delaying egg laying (Kirschvink 2000; Chatterji and Shaharom 2009). Our toads appear to have abandoned spawning because of the impending EQ and then delayed spawning again until after the end of the EP. Toads at this site show lunar periodicity with most mating occurring close to the full moon (Grant et al. 2009). Hence the spawning of *B. bufo* 10 days before and 11 days after April's full moon is unusual, and toads may well have shifted the

timing of breeding due to the high seismic activity around the full moon period when they would normally have spawned. If this is the case, toads show considerable plasticity in breeding phenology. Amphibians are ectothermic and have permeable skins; consequently, their activity is normally highly dependent on weather, particularly rainfall and temperature (Beebee and Griffiths 2000). No unusual weather occurred in the days leading up to the EQ that could have caused toads to abandon spawning and leave the breeding site and toad numbers were not correlated with any of the climatic variables tested. There are several possible mechanisms by which the prediction of seismic events by animals may occur. P-waves, EQ lights and ground tilt occur seconds to minutes before EQs (Kirschvink, 2000) but the toads in this study changed their activity 5 days before the main seismic event, so these are unlikely to be the cause. Humidity can rise before EQs due to raised groundwater levels, but *B. bufo* is not particularly affected by humidity (Gittins et al. 1980; Sinsch 1988). Increased electrical activity is a possible cue but electrical sensitivity in terrestrial animals is generally low due to the high electrical resistance of air (Kirschvink 2000). Toads could have been responding to a foreshock of magnitude 4.3 that occurred on the afternoon of 30/3/09, but single and amplexed toad numbers did not decline to unusual levels until 3 days after the foreshock,

so this is unlikely. Alternatively, EQ prediction may occur because of changes in the Earth's magnetic field (Kirschvink 2000). Geomagnetic anomalies occurred a few days before the M=7.0 1978 Alay EQ Shapiro and Abdullabekov (1982) and the 1989 M=7.1 Loma Prieta EQ (Fraser-Smith et al., 1990; Bernardi et al. 1991). Toads have been shown to be responsive to geomagnetism in orientation and homing experiments (Sinsch 1992). The presence of magnetoreceptors in nocturnal animals for use in navigation and circadian systems may enable them to detect small changes in geomagnetism and hence predict and evade impending EQs (Kirschvink 2000). Newts and salamanders are known to have particularly sensitive magnetoreceptors (Phillips, 1986 a,b; Phillips et al. 1995) and, if magnetoreception is the means by which amphibians are able to predict seismic events, their behaviour could be a good predictor of EQs.

A further possible precursory phenomenon that could be detected by toads is anomalies in levels of radon gas in groundwater. Amphibians are closely connected with the aquatic environment and are sensitive to changes in water chemistry (Vitt et al. 1990). Radon levels increased 10-fold 9 days before the Kobe, Japan EQ (1995, M=7.2) (Igarashi et al. 1995) and there have been numerous other reports of similar radon anomalies before

large EQs. There were also anomalies in uranium (a radon precursor) levels for some months before the L'Aquila EQ (Plastino et al. 2010). It is clear from Figs 6 and 7 that ionospheric perturbations before the EQ coincide with toads apparently leaving the breeding site. Both VLF propagation paths passing near the epicentre show disturbances 5 days before the EQ. What is less clear, however, are the factors causing both the ionospheric disturbances and the reduced numbers of toads at their breeding site. Lithosphere–atmosphere–ionosphere coupling hypotheses have been proposed including the release of radon gas before EQs changing atmospheric conductivity and thereby modifying atmospheric electric fields and currents (Grimalsky et al. 2003; Sorokin et al. 2005). Another possibility for lithosphere–ionosphere coupling is the release of gravity waves caused by pre-seismic gas and water release at the ground surface, giving rise to changes in air density and the distribution of charged particles. Gravity waves can be exponentially amplified as they gain in altitude, due to the conservation of kinetic energy and the corresponding exponential decrease in atmospheric density (Artru et al. 2005). Gravity waves can therefore cause perturbations in the ionosphere (Molchanov et al. 2001, 2004) and probably explain the VLF signal anomalies in 10 EQs analysed by Molchanov and Hayakawa (1998). Observational evidence has

shown that gravity waves can be the cause of LF anomalies before and after EQs, by the analysis of signal amplitude and phase inside the Fresnel zone of the Japan Kamchatka wavepath over the course of three large EQs (Rozhnoi et al. 2007). Whatever the ultimate cause of the ionospheric disturbance, the evidence presented here supports the ability of the common toad to detect and respond to this cue, thereby showing a seismic predictive response commonly thought to exist in animals, but rarely documented scientifically. The reason many reports of unusual behaviour before EQs lack scientific credibility is that they were made after the event but a feature of this study is that toads' activity and breeding behaviour was recorded before, during and after the event. Many reports of unusual behaviour do not consider other factors such as weather that may have affected the animals and are unable to link unusual behaviour to any other extraordinary geophysical phenomena. As most reports of unusual animal behaviour before EQs occur only a short time before the EQ and very near the epicentre (Buskirk et al. 1981), the data presented here are particularly interesting in that our toads were apparently able to anticipate an EQ over 75 km away, several days in advance.

The data we observed in toads differs from that seen in ants, mice and birds before EQs. Ants and some species of birds showed no response before a strong EQ, and other species of birds showed a response several minutes before the event. Rodents' responses occurred one to three days before the EQ. Although the examples discussed here are isolated examples which have not been reproduced, and therefore must be interpreted with caution, it seems likely that the level and nature of seismic anticipatory responses in animals are taxon specific and adaptive. Ants' small size and burrowing habits may mean that they are in little danger from EQs, and birds are able to leave the affected area quickly by taking flight. Toads, however, are very slow moving animals, which would need hours or days to find safe areas so there would be more evolutionary pressure on them to develop an effective early seismic escape response.

Whether toads would exhibit similar behaviour at other locations and preceding other large seismic events remains to be seen but the results reported here suggest that toads detect whatever phenomenon is giving rise to the perturbations in the ionosphere and are thus able to anticipate seismic events. Testing this hypothesis would, however, be very difficult, given the rarity and unpredictability of EQs.

Further investigation has led to the proposal of a hypothesis, based on water chemistry changes, for the mechanism by which toads and other aquatic and semi-aquatic animals might change their behaviour before earthquakes. This has been published as: Grant RA, Halliday T, Balderer WP, Leuenberger F, Newcomer M, Cyr G and Freund FT. 2011. Ground water chemistry changes before major earthquakes and possible effects on animals. International Journal of Environmental Research and Public Health 8(6):1936-56.

Chapter 5: Natural variations in geomagnetic fields and their effects on amphibian breeding migrations and reproduction

5.1 Introduction

The Earth's magnetic field is known to influence many organisms (Kirschvink and Gould 1981), and some amphibians are sensitive to geomagnetism (Phillips et al. 1995, 2002; Phillips 1986a,b; Fischer et al. 2001; Deutschlander et al. 1999b). Previous work (Grant et al. 2009) suggested that newts' breeding phenology may be connected to magnetic fields, as newts in two separate studies appeared to avoid arriving at breeding ponds during the third quarter of the lunar cycle, when geomagnetism is highest. Some species of amphibian use magnetic cues in orientation towards the breeding site and in homing when displaced from the breeding pond (Phillips 1986a, b; Sinsch 1987; Phillips and Borland 1992a, b; Phillips et al. 1995; Deutschlander et al. 1999a, b, 2000; Phillips et al. 2001; Diego-Rasilla et al 2005).

It appears, however, that there has been no research on the effects of magnetic fields on amphibian reproductive behaviour or breeding phenology. Similarly, I was unable to find any reports of amphibians' response to natural variations in the Earth's magnetic field, such as geomagnetic storms, although other animals have been shown to be affected (Larkin and Keeton 1976; Vanselow and Ricklefs 2005; Esquivel et al. 2007). If cues from the geomagnetic field are used in amphibians' migrations towards their breeding sites, migration could be disrupted during fluctuations of the field such as geomagnetic storms, which would affect the numbers of amphibians arriving. This study aimed to test the hypothesis that amphibians' breeding migrations would be affected (i.e. fewer arrivals) when the geomagnetic field is disturbed, by looking at arrivals in relation to the K-index, an index of magnetic disturbance.

The Earth's magnetic field approximates to a magnetic dipole, aligned 11.3° away from the Earth's axis of rotation (Demorest, 2001) (Figure 33).

Although it was initially believed that the field resulted from a permanent magnetism at the poles, studies of paleomagnetic samples show that the magnetic field of the Earth is dynamic rather than static, and has reversed

several times. It is now accepted that Dynamo Theory best explains the maintenance of the field (Demorest, 2001). The Earth's core is primarily made of iron, of which the outer part is liquid, at a temperature of 4000K. A conductive fluid (such as molten iron) moving through a magnetic field will induce currents, which in turn influence the motion of the fluid and thereby cause a further magnetic field to be induced (Demorest, 2001).

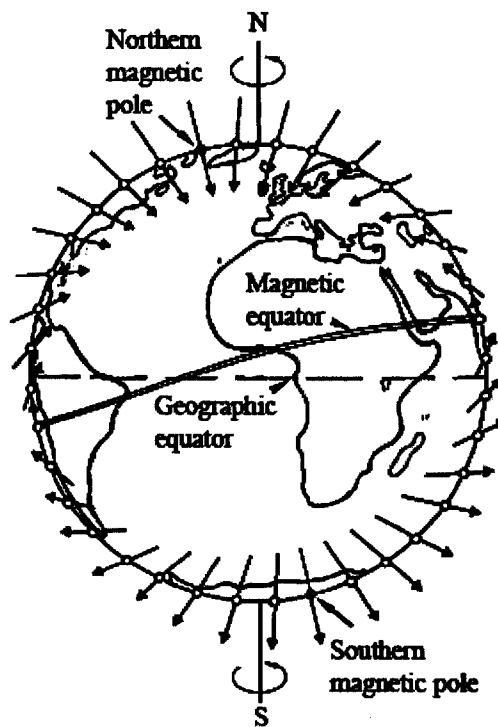


Figure 33: Schematic of the Earth's magnetic field. The arrows show the direction of the field. From Wiltchko and Wiltchko (1996).

The magnetic field is a three dimensional vector (i.e. it has both magnitude and direction). The strength of the field at the Earth's surface ranges from less than 30 microteslas (0.3 gauss) in an area including most of South America and South Africa to over 60 microteslas (0.6 gauss) around the magnetic poles in northern Canada and southern Australia, and in part of Siberia. The magnetic field is subject to fluctuations, usually caused by space weather such as solar flares. Magnetic storms are comparatively infrequent global perturbations of the geomagnetic field, usually lasting a few days. The frequency of magnetic storms depends to an extent on the timing of solar flares but is in the region of 10 per year for small events, with larger storms being much rarer (Love & Gannon 2009). The scale of variations caused by magnetic storms is in the region of hundreds of nanoteslas (Silbergleit 1999; Burch 2001), which is of a similar order of magnitude to spatial variations (Semm and Beason 1990; Walker et al. 1992, 2002; Fischer et al. 2003). A magnetic threshold sensitivity of 10 to 50 nT has been shown experimentally in homing pigeons, and is also known in sharks and whales (Walker et al. 2002). Therefore it is possible that animals that use the magnetic field in navigation and homing may be confused by the variations in geomagnetism caused by magnetic storms (Vanselow and Ricklefs 2005).

The K-index is a measure of magnetic field disturbances on a scale of 0-9, with 1 indicating calm, and 5+ indicating a geomagnetic storm. The Kp index is an official worldwide index based on a weighted average of the data from many observatories. Migratory animals are known to be affected at Kp levels of 5 and above (National Oceanic and Atmospheric Administration

<http://www.swpc.noaa.gov/NOAAscales/#GeomagneticStorms>).

Wiltschko and Wiltschko (1972) first proposed that birds are sensitive to the Earth's magnetic field. This was followed by evidence that bees are also affected by geomagnetism (Martin and Lindauer, 1977). Since then, magnetic field sensitivity has been shown in a variety of animals including deer, cows, bats, mole rats, hamsters, mice, and that it is generally used in navigation and homing (Olcese et al. 1985, Burda et al. 1990, Kimchi and Terkel 2001, Deutschlander et al. 2003, Muheim et al. 2006, Thalau et al. 2006; Begall et al. 2008, Holland et al. 2008).

Amphibians have also been found to be influenced by the Earth's magnetic field, in homing and navigation experiments. Sinsch (1987) displaced toads

(*Bufo bufo*) from their breeding ponds and, by glueing a bar magnet to the toads' heads, caused a significant number of them to orient randomly rather than towards the breeding site, when compared to the control group where all toads oriented towards the breeding site. Sinsch (1987) concluded that toads use olfactory and magnetic cues to re-orient themselves after displacement. Fischer et al. (2001) found that the eastern red-spotted newt (*Notophthalmus viridescens*) oriented in its home direction when displaced 45 km NNE of its home pond. When the magnetic field around the newts was manipulated to resemble that on the opposite side of the home pond (a 2° increase in magnetic declination) newts reversed their direction, providing evidence that these newts use the Earth's magnetic field for orientation.

The newt, *Notophthalmus viridescens*, uses directional information from the magnetic field of the Earth to orient to its home shore (Phillips, 1986a, b; Phillips and Borland 1992a, b; Deutschlander et al 1999a, b, 2000; Phillips et al. 2001) but is also capable of “true navigation” i.e. the use of positional, rather than just orientational information (Phillips et al. 1995). As the home range of newts is small (only 2-3 km at most (Phillips et al. 1995)), newts must be able to detect tiny fluctuations in the field or changes

in total intensity of approximately 0.01–0.001% of the ambient field (Phillips et al. 2002).

Alpine newts (*Triturus alpestris*) also appear to use the Earth's magnetic field in homing (Diego-Rasilla et al. 2005). When skies are overcast newts use a magnetic compass to orient. Light from the moon appears to disrupt their magnetic orientation, however, and they align significantly along the moon's azimuth. This could be a response to a celestial compass or an artefact, e.g. newts responding to another factor such as the light and shade pattern caused by moonlight.

Phillips and Borland, (1992a) and Deutschlander et al. (1999a, b) have reported that, in newts, the magnetic orientation response becomes shifted by 90 degrees in long wavelength (>500 nm) light, suggesting that magnetoreception is dependent on light. The presence of a similar response to long wavelength light in bullfrog tadpoles suggests a common mechanism in both anurans and urodeles and, from this, it can be inferred that the light-dependent magnetic compass response evolved early in amphibians (Freake and Phillips 2005). The precise mechanism by which light-dependent magnetoreception is mediated is not fully understood but

there are several hypotheses, of which the electron pair hypothesis has gained support in recent years. A detailed review of the possible biochemical mechanisms relating to the electron pair hypothesis can be found in Rodgers and Hore (2009).

It has been shown that there are two main mechanisms of magnetoreception in animals.

1. A magnetite-based positioning system, providing a map component, which indicates position but not direction (Phillips et al. 2010). Magnetite is a ferromagnetic mineral with chemical formula Fe_3O_4 , and has magnetic properties, such as attracting small pieces of iron.
2. A radical pair mechanism, which is light-dependent and provides directional information (a compass) (Phillips et al. 2010).

There is behavioural and neurological evidence that amphibians use the light-dependent magnetic compass (LDMC) (Phillips and Deutschlander, 1997), and, in newts, light is received by the pineal organ (Deutschlander et al. 1999a, b. Phillips et al. 2001). Later it was found that a non-light-dependent magnetite-based map component also exists which is able to give positional, not directional information (Freaker et al. 2006,

Winkelhofer and Kirschvink 2010). It is so far rather a mystery why two systems have evolved, particularly when the magnetite-based system could have evolved to supply positional information, and has indeed done so in some animals such as blind mole rats which live underground (Burda et al. 1990; Marhold et al. 1997, Kimchi and Terkel 2001, Kimchi et al. 2004, Thalau et al. 2006, Némec et al. 2007). Phillips et al. (2010) suggest that the light-dependent radical pair mechanism might produce a three-dimensional light and colour response imposed on the visual surroundings acting as a spherical co-ordinate system, and the magnetite-based system is then specialised for providing map positioning information.

Although there are many examples in the literature where the magnetic field surrounding animals has been experimentally manipulated (e.g. Åkesson, 1994; Mouritsen, 1998), the question of whether animal behaviour varies in relation to natural variations such as magnetic storms has hardly been addressed. There are a few examples of magnetic disturbance affecting several species, particularly birds. Larkin and Keeton (1976) looked at pigeons which had bar magnets or brass bars (controls) strapped to their backs. Control birds were affected by variations in magnetic field (the K-index) but birds carrying the magnets were not,

indicating that pigeons are affected by natural variations in the K-index, but that this can be masked by proximity to magnets. Sperm whale strandings in the North Sea are thought to be related to solar activity and / or geomagnetic storms (Vanselow and Ricklefs 2005). Esquivel et al. (2007) looked at the effects of varying magnetic field on the honeybee *Schwarziana quadripunctata*, and found that the experimentally-applied magnetic fields did not affect behaviour but that a geomagnetic storm that occurred during the experimental period did have a statistically significant effect on behaviour.

I aimed to test the hypothesis that naturally-occurring geomagnetic disturbances will affect the numbers of migrating amphibians, and/or alter reproductive behaviour such as the number of amplexed and / or spawning amphibians, by comparing data already collected on amphibian arrivals and reproduction with the local K-index for each day.

5.2 Methods

5.2.1 *Amphibian arrivals and reproduction data*

To investigate the effect of natural magnetic variation on amphibian arrivals and reproduction, five datasets were used, collected at various locations:

1. 74 days of arrivals data over the breeding season in 2000 and 2001, of the common toad, *Bufo bufo* collected by E. Chadwick at Llysdimam Pond. Males, females and amplexed pairs were analysed separately.
2. 205 days of arrivals at Marston Pond from 1978-1989 of *B. bufo* collected by Tim Halliday. Males, females and spawning pairs were analysed separately.
3. 51 days of arrivals at Marston Pond from 1979-1985 of the frog *Rana temporaria* collected by Tim Halliday. Males, females and spawning pairs were analysed separately.
4. 44 records, data collected by myself at San Ruffino Lake in 2007. Details of the methods of data collection have been given earlier in chapter 2. Data

from 2006 were not used in the analysis as they were not differentiated by sex. Males, females and amplexed pairs were analysed separately.

5. Continuous data on newt arrivals (the palmate newt, *Lissotriton helveticus* and the smooth newt *Lissotriton vulgaris*) at Llysdimam pond from 10/1/1992 to 30/9/2002 except 1994 and 1996 collected by E. Chadwick and others at Llysdimam field centre. After removing days where no data were collected or the traps were not checked, there were 1142 records. Animals were collected using pitfall traps and the pond was surrounded by a drift fence (Chadwick et al. 2006). Males and females were analysed separately.

A drawback of using data collected on animals at the breeding site is that they have already completed their migration, and therefore will probably not be using magnetic cues at this point. Schmidt-Koenig and Walcott (1978) found that visually-impaired pigeons could use the Earth's magnetic field to arrive approximately 0.5 to 5 km from their loft, but used vision to home in on the loft during the final few kilometers. Magnetic field fluctuations are likely to affect amphibians during their migration, but their reliance on geomagnetism may wane as they approach the breeding site, so any geomagnetic effects may occur in the days prior to their arrival. Toads

are known to migrate from up to 1.6 km (Sinsch, 1988) from their overwintering refugia and newts have been found up to 146m from the breeding pond and migrate with a median speed of 4.6 miles/hour, being able to cover 137 m in a single night (Jehle and Arntzen, 2000), therefore the newt breeding migrations could be expected to take at least one night, and toads possibly longer as they may be further from the breeding site. I therefore lagged the K-index by one and two days, so that the effects of the K-index on animals before they arrived at the breeding site could be analysed, by using the K-index from the previous day (K+1), or the day before that (K+2) (as well as the current day) in the analysis. A limitation of this procedure is that it makes the assumption that amphibians move directly to the breeding site, whereas in fact, particularly in the case of newts, migrations are not highly synchronised and animals may linger in refugia close to the breeding site for a number of days (Jehle and Arntzen, 2000). In this case, they may use only local cues to make their final approach to the breeding site, the main migration having been completed earlier.

5.2.2 *Magnetic field data*

The following information on the Earth's magnetic field was taken from the website of the National Geophysical Data Centre

(<http://www.ngdc.noaa.gov/geomag/geomag.shtml>) which is part of the National Oceanic and Atmospheric Administration (www.noaa.gov) and was accessed on 11.11.2009. Each magnetic observatory produces its own K-index which is a quasi-logarithmic index of local geomagnetic activity taken at 3-hourly intervals. The K-index is a relative scale, and its intensity is relative to a magnetically quiet day at that particular observatory and is measured on an integer scale, 0-9. The Kp index is a mean of the K-index taken from 13 observatories between 44° and 60°N and 44° and 60°S, which is less useful as it will obscure local effects. Therefore the K-index was used in this study. The values of the K-index for the nearest observatories to the breeding sites were obtained. As the K-index is always recorded at eight 3-hourly intervals, a mean value was calculated for each day. The nearest geomagnetic observatory to the San Ruffino Lake site is at L'Aquila (Observatory code AQU; 42.3833°, 13.3167°) (approximately 75 km from the breeding site). K-indices were downloaded from the data

portal at the L'Aquila observatory from 2006 to 2009 (inclusive). For the UK K-index data, the observatory at Hartland was used (Observatory code HAD; 50.9950°, 355.5160°) as this was the nearest observatory to all three UK sites: Marston pond, Llysdimam pond and Llandrindod Wells Lake. Llysdimam Pond and Llandrindod Wells Lake are approximately 160 km from Hartland, and Marston Pond is approximately 245 km from Hartland. Again, a mean value from the 8 daily readings was obtained for each day.

5.2.3 Statistical analysis

The K-index (unlagged, and lagged by 1 and 2 days) was used as a predictor variable in linear regression analysis. Each site, species and sex were analysed separately to look for differences in their response to magnetic fields. For analysis, the log to the base ten of anuran numbers was used in order to normalise the residuals. To deal with zero values, I followed the convention of adding 0.5 to all values (Macdonald 2009). For newts, none of the usual transformations (log, square root, cube root, reciprocal etc.) were able to normalise the residuals as the data were highly skewed to the left. Therefore the dependent variable (number of newts arriving) was rank transformed in order that the data would not violate the

assumptions of linear regression. Rank transform renders the regression non-parametric and frees it from assumptions of normality. However, there is a loss of statistical power and inflated Type I error rates with this procedure (Headrick and Routou 2001). Additionally, to look for thresholds in the data, they were also analysed categorically. Mean amphibian (urodele or anuran) arrivals per day were calculated by taking the total number of animals that arrived over the entire study period and dividing by the number of days. For each K-index category, the expected number of animals was calculated by taking the number of days in each category and multiplying by the mean number of animals per day. For newts, the two days when K was greater than 5.0 were included in the final category; $K > 4.0$. Then a chi-squared test was carried out to see whether observed numbers were significantly different from expected numbers in each K-index category.

5.3 Results

5.3.1 Anurans – Regression analysis against the K-index

There was no significant relationship between the number of male, female or amplexed / spawning anurans at any of the breeding sites and the intensity of geomagnetic disturbance when the K-index was not lagged, in either *Bufo bufo* or *Rana temporaria* (Table 9). In order to explore this further the raw data were examined where the K-index was 5 or greater, indicating a geomagnetic storm (Table 10). It can be clearly seen that several very large arrival events occurred during geomagnetic storms. In fact, the day of the largest arrival of *Bufo bufo* in 2001 at Llandrindod Wells Lake occurred during a very strong ($K > 7$) magnetic storm on 31st March 2001. In case this indicated a preference for arrival during magnetic storms, the largest arrival for 2000 at the same breeding site was inspected. This occurred during a period of quiet magnetic activity ($K = 0.6$; 13th March 2000; 663 toads arrived at the site). During the geomagnetic disturbance of 31st March 2001 recorded at Hartland Observatory, there were also high numbers of amplexed pairs arriving at Llandrindod Wells Lake. On 4th April 1988 there was a minor ($K = 5.1$) magnetic storm and there were large arrival and spawning events at Marston Pond on this date. Equally, many large arrival and spawn events occurred at Marston Pond on magnetically quiet days (Table 9).

Table 9. Statistical outcomes for geomagnetic field analysis (anurans).

Regression of numbers of amphibians against the K-index

<i>Bufo bufo</i> Llandrindod Wells Lake	N	R² (adj)	P value
logten males v K-index	74	5.40%	0.026
logten males v K+1	74	7.10%	0.012
logten males v K+2	74	2.50%	0.096
logten females v K-index	74	2.20%	0.109
logten females v K+1	74	5.90%	0.021
logten females v K+2	74	1.70%	0.136
logten pairs v K-index	74	0.80%	0.215
<i>Bufo bufo</i> San Ruffino Lake	N	R² (adj)	P
logten males v K-index	44	4.70%	0.085
logten males v K+1	44	4.70%	0.084
logten males v K+2	44	0.00%	0.341
logten females v K-index	44	0.00%	0.696
logten females v K+1	44	0.00%	0.594
logten females v K+2	44	5.30%	0.073
logten pairs v K-index	44	1.80%	0.189
<i>Rana temporaria</i> Marston Pond	N	R² (adj)	P
logten males v K-index	51	0.00%	0.939
logten males v K+1	51	12.70%	0.006
logten males v K+2	51	2.00%	0.16
logten females v K-index	51	0.00%	0.909
logten females v K+1	51	8.10%	0.025
logten females v K+2	51	1.50%	0.193
logten pairs v K-index	51	0.00%	0.894
<i>Bufo bufo</i> Marston Pond	N	R² (adj)	P
logten males v K-index	205	0.00%	0.471
logten males v K+1	205	0.80%	0.111
logten males v K+2	205	1.20%	0.067
logten females v K-index	205	0.00%	0.536
logten females v K+1	205	0.00%	0.81
logten females v K+2	205	0.00%	0.417
logten pairs v K-index	205	0.00%	0.314

Table 10. The numbers of arriving anurans and the numbers of paired animals at the breeding site during geomagnetic storms ($K > 4$)

Date	Species	Site	K-index	Number of males	Number of females	Spawning /amplexed (pair)
6-Apr-00	B.bufo	Llysdynam	4	0	0	0
10-Apr-82	B. bufo	Marston	4	0	0	6
14-Mar-81	R. temp	Marston	4	1	1	1
01-Apr-07	B.bufo	San Ruffino	4	70	0	1
11-Apr-01	B.bufo	Llysdynam	4.125	22	2	0
27-Mar-78	B. bufo	Marston	4.375	2	1	0
01-Apr-89	B.bufo	Marston	4.375	2	1	13
07-Apr-00	B.bufo	Llysdynam	4.5	0	0	0
31-Mar-89	B.bufo	Marston	4.75	1	1	0
20-Mar-01	B.bufo	Llysdynam	5	0	0	0
04-Apr-88	B.bufo	Marston	5	123	12	1
29-Mar-89	B.bufo	Marston	5.125	2	2	6
31-Mar-01	B.bufo	Llysdynam	7.125	454	41	30

Additionally, none of the lagged K-index variables had a significant association with arriving anurans after Bonferroni correction for multiple testing was applied. However there were several outcomes which were close to significant, for example arriving male *Rana temporaria* at Marston Pond and arriving male *Bufo bufo* at Llandrindod Wells Lake. These were both regressed against the K-index, lagged by 1 day.

5.3.2 *Urodeles* - regression against the K-index

There was no significant relationship between the number of arriving newts of either sex or species, and the K-index (Table 12). Examination of the data showed that, unlike the anuran data set, there were very few days when geomagnetic disturbance occurred. There were only two occasions when the K-index was five or greater, which occurred on 24/5/2000 and 6/11/2001, where the K-index was 5.125 and 5.625 respectively. One newt arrived on 25/4/2000 and no newts arrived on 6/11/2001, but this sample size (N=2) is too small to carry out statistical analysis.

Table 12. Statistical outcomes for geomagnetic field analysis (newts)

K-index lag	Species	Sex	N	R-Sq(adj)	P value
0 days	palmate newt	male	1142	0.00%	0.787
1 day	palmate newt	male	1142	0.00%	0.736
2 days	palmate newt	male	1142	0.00%	0.914
0 days	palmate newt	female	1137	0.00%	0.708
1 day	palmate newt	female	1137	0.00%	0.636
2 days	palmate newt	female	1137	0.00%	0.870
0 days	Smooth newt	male	1138	0.00%	0.926
1 day	Smooth newt	male	1138	0.00%	0.772
2 days	Smooth newt	male	1138	0.10%	0.189
0 days	Smooth newt	female	1134	0.00%	0.915
1 day	Smooth newt	female	1134	0.00%	0.431
2 days	Smooth newt	female	1134	0.00%	0.954

5.4 Discussion

5.4.1 *Anurans*

It appears that, in the anurans I studied (*B. bufo* and *R. temporaria*), the extent of geomagnetic disturbance does not significantly affect migration and breeding activity, when analysed by regression on the K-index. The high sensitivity of many amphibians to magnetic fields makes this result surprising. When considering the non-lagged K-index, there were several very large arrival events during strong magnetic storms and similarly there were large arrivals during geomagnetic quiet days. When considering the lagged K-index, which takes into account the fact that magnetic cues may only be used further from the breeding site, the results were non-significant after Bonferroni correction. The only results that came close to significance were those where the K-index was lagged by one day. From the Chi-squared analysis it appears that arrivals are nonrandom with respect to K-index category. However, the differences are small (Fig. 34). It is possible that because of a weak biological effect, and the use of the Bonferroni

correction, my analysis was not powerful enough to detect the effect of geomagnetic storms, should this exist. Alternatively, as explosive breeders such as *Bufo bufo* and *Rana temporaria* have a large endogenous component (i.e. an internally generated physiological change which is not dependent on external cues) to breeding phenology (Heinzmann 1970; Wells 1979; Sinsch 1988) it is possible that, once breeding has started, even a strong geomagnetic storm is not able to interrupt breeding.

5.4.2 *Urodeles*

Regression analysis showed that the numbers of arriving newts were not correlated with the intensity of geomagnetic storms. However there were only two days where the K-index was 5 or greater, which is not sufficient to determine whether magnetic storms affect urodele activity. Chi-squared analysis shows that fewer newts arrived when the K-index was above four. Certainly, unlike anurans, there were no large arrival events at all when the K-index was greater than 4, and the frequency of arriving newts was lower than expected from $K > 3.1 < 4$ and far fewer than expected above $K = 4.1$. This suggests that newt migrations are affected by high levels of geomagnetic disturbance, but further investigation, particularly on days of

high geomagnetic activity would be needed to clarify this. More newts than expected arrived during $K > 1 < 2.1$, suggesting that newts prefer to move during a low level of geomagnetic disturbance.

In summary, it appears that geomagnetic storms do not significantly affect amphibians during their breeding migrations. However, the loss of statistical power by the use of rank-transform regression and Bonferroni correction means that weaker effects will not be detected. It is possible that anurans are affected by geomagnetic storms when they are 1 day away from the breeding site but further analysis would be required to confirm this. The lack of geomagnetic disturbance during the period over which newt arrivals were recorded, means that no firm conclusions can be drawn about the effects of geomagnetic storms on urodeles.

Chapter 6: Modeling amphibian breeding phenology

6.1 Introduction

In the previous chapters, various geophysical variables (moon phase, seismic activity and geomagnetism) have been considered in relation to amphibian arrivals and reproduction. Considering each variable on its own, however, does not show the relative contribution of each of the variables to the variation in numbers of arriving amphibians. It also does not take into account how other variables such as weather fit into the picture. In order to evaluate the relative contribution of these variables to variation in timing of arrival and reproduction, it is necessary to consider them simultaneously using regression-based statistical models. In this way, one can attempt to build a fuller picture of the interacting variables that may affect amphibians arriving at their breeding site, and once a statistical model has been constructed, it can then be used to attempt to predict amphibian arrivals. Another reason for wishing to use multivariate regression-based models is that in previous chapters, effects in the data may have been lost due to lack of statistical power. Using regression-based multivariate techniques

removes problems of non-normally distributed data, as appropriate distribution models (e.g. Poisson) can be selected to deal with the probability distribution. Considering all variables simultaneously removes necessity of corrections for multiple testing and their resulting loss of statistical power.

Most previous studies use linear regression-based approaches to model parameters related to reproduction in amphibians. Henzi et al. (2005) used a suite of environmental variables to model chorus attendance in Painted Reed Frogs (*Hyperolius marmoratus*), a prolonged breeder, using generalised linear models with a Poisson distribution. Males and females responded to different combinations of variables and the model was able to predict 87.78% of the variance in chorus attendance in females and 76.6% for males. Banks & Beebee (1986) were able to predict breeding of the Natterjack toad (*Bufo calamita*), which breeds sporadically over a prolonged period from March to July. Banks & Beebee (1986) used discriminant analysis with a range of climatic variables to achieve predictions of 72-75% accuracy for calling, and 80% for spawning. All of the above examples relate to prolonged breeders. The breeding phenology of explosive breeders may be more difficult to model as it is

generally thought to be less dependent on environmental variables with a larger endogenous component (Heinzmann 1970; Wells 1979; Gittins 1983; Sinsch 1988; Reading 1998; Oseen & Wassersug 2002).

In this chapter, I will consider the properties of the data collected, and select appropriate models based on the properties of the data. This will enable an evaluation of the contribution of each variable to the model and allow an assessment of the statistical significance of each. The results will be discussed and I will then use these models to attempt to predict amphibian arrival in single years, based on the models constructed.

6.2 Methods

Methods of data collection have been described in previous chapters, in particular sections 2.2.1 to 2.2.6. Some additional variables which may be important in influencing amphibian breeding phenology, namely daylength (photoperiod) (Canavero and Arim 2009) and degree days (Beattie 1985; Timm et al. 2007), were also used. The times of sunrise and sunset for the city of Ancona (43°3'N 13°30'E) for the San Ruffino breeding site, and the city of London (51° 32'N, 0 ° 5'W) for the UK breeding sites were obtained

from the US Naval Observatory's database on 4.9.10.

(<http://www.usno.navy.mil/USNO/astronomical-applications>). These data were used to calculate daylength for each day. Degree days are a measure of thermal heating and are calculated by subtracting a base temperature from the average daily temperature- $(T_{MIN} + T_{MAX}/2)$ -base temperature. The base temperature was set at 4°C for both species as this is one of the threshold temperatures reported for breeding migrations for *B. bufo* (Gittins, 1980). For *R. temporaria* the temperature range is thought to be between 3.1°C and 7°C (Beattie 1985).

6.2.1 Variables

In this analysis, there were eight predictor variables (Table 14) plus a dummy variable (indicator variable) which was included to account for differences in breeding site, which is a fixed effect (SITECODE). The three dependent variables were the number of males arriving (MALES), the number of females arriving (FEMALES) and the number of amplexed or spawning pairs recorded (PAIRS).

Table 14. The predictor variables used in this analysis, and their abbreviations.

Variable	Abbreviation
Daylength / photoperiod	DAYL
Number of days to a past or future full moon	MOON15
The cosine of the number of days since the past full moon (in radians)	COSMOON29
Maximum temperature in degrees Celsius	TMAX
Minimum temperature in degrees Celsius	TMIN
Rainfall in mm	PREC
Degree-days	DEGD
K-index	KIND
Site	SITECODE

6.3. Theoretical background and analysis

Statistical analysis of the datasets was carried out using multivariate circular-linear negative binomial regression using the software Stata 11.1 from, StataCorp LP, 4905 Lakeway Drive, College Station, Texas 77845, USA (www.stata.com). Pearson correlations were carried out using Minitab 15 from Minitab Ltd., Brandon Court, Unit E1-E2, Progress Way, Coventry CV3 2TE, UK.

6.3.1 *Summary of the data*

A summary of the data to be analysed is shown in Tables 15 and 16. In total there were six datasets to be analysed: 1. Male toads; 2. Female toads; 3. Paired toads; 4. Male frogs; 5. Female frogs; 6. Paired frogs. For each dataset the total number of observations or cases in the dataset was calculated (N), the mean number of animals arriving (Mean), the standard deviation (Std. Dev.), the minimum number of arrivals in each dataset, which in all cases was zero (Min) and the maximum number arriving (Max). Finally, the variance was calculated for each of the six datasets (Variance).

Table 15. Summary of the data – *Bufo bufo*

Variable	Observations (N)	Mean	Std. Dev.	Min	Max	Variance
Males	316	27.8	53.5	0	505	2862.25
Females	316	3	5.4	0	29	29.16
Pairs	316	4.1	9.2	0	72	84.64

Table 16. Summary of the data – *Rana temporaria*

Variable	Observations (N)	Mean	Std. Dev.	Min	Max	Variance
Males	62	6	2.1	0	58	4.41
Females	62	4.7	7.9	0	31	62.41
Pairs	62	4.1	9.2	0	40	84.64

6.3.2 Count data

Amphibian arrival data are count data, and therefore do not follow a normal distribution (Cameron and Trevedi 1998). While count data can be transformed to approximate a normal distribution by using, for example, log or square root transformations, this is not recommended (O’Hara and Kotze 2010). In particular this is not a valid strategy in this case because there are many zero values; both the log and square root of zero are undefined, hence the case for using data transformation is considerably weakened. The problem can be somewhat overcome by using ranked variables, thus rendering the regression nonparametric and I tried this approach in chapter 5. However the results were inconclusive, possibly due to the loss of statistical power and high probability of type 1 errors due to this approach (Headrick and Routou 2001).

6.3.3 Poisson and Negative Binomial Distributions

A Poisson distribution is the benchmark model for count data (Cameron and Trevedi, 1998) but Poisson distribution assumes that the mean is equal to the variance. Where the variance is considerably greater than the mean a negative binomial distribution (NB) is more appropriate. The NB corrects for overdispersion (i.e. the presence of greater variability than is assumed in a Poisson model). The mean and variance were calculated for the datasets and the variance was found to greatly exceed the mean in all except one set (Tables 15 and 16), indicating that a negative binomial model should be used. The alpha value (which is the coefficient of overdispersion) can be inspected for NB models. A zero value of the alpha coefficient indicates that the model is better estimated using a Poisson model.

The general equation for negative binomial regression is:

$$\text{Natural log}(Y) = \text{Intercept} + b_1(X1) + b_2(X2) + b_3(X3) \dots$$

This implies:

$$Y = \exp(\text{Intercept} + b_1(X1) + b_2(X2) + b_3(X3)) \dots$$

$$= \exp(\text{Intercept}) * \exp(b_1(X1)) * \exp(b_2(X2)) * \exp(b_3(X3)) \dots$$

6.3.4 *Maximum likelihood analysis and interpreting coefficients*

Maximum likelihood is a method of estimating the parameters of a model and uses an iterative approach to find the parameters that make the probability of the observed results the most likely. In fact the maximum likelihood method of fitting a regression line to multivariate data is always the correct one, although if the data are normally distributed the commonly used “least squares” method gives an identical result (Sokal & Rohlf 1995). Therefore, for non-normally distributed data, maximum likelihood estimation is the method of choice. As the method is complex for multivariate data, and relies on an iterative approach, the calculations can only be carried out by computer (Sokal & Rohlf 1995). Once the algorithm used by the computer for estimating the parameters of the model has successfully found a solution, this is known as convergence (Gould et al. 2006). However, sometimes there will be no acceptable solution and in this case the model will fail to converge, meaning that the algorithm was unable to find values for the parameters that make the observed result likely (Gould et al. 2006). In this case, variables can be dropped to enable the algorithm to converge. The tables show the estimated negative binomial regression coefficients for the model. As the dependent variable is a count

variable, NB regression models the log of the expected count as a function of the predictor variables. Therefore for a one unit change in the predictor variable, the difference in the logs of expected counts is expected to change by the respective regression coefficient, given the other predictor variables in the model are held constant (Gould et al. 2006).

6.3.5 *Serial Dependency*

An additional issue is the possibility of serial dependency in the data, i.e whether the presence of a number of amphibians on a given night influences the probability of the number of arrivals on a subsequent night (also known as autocorrelation). With the amphibian arrivals data, animals were intercepted and counted as they made their way towards the breeding site. Therefore it is unlikely that the same animals would be counted on subsequent evenings, as they would not return that way until the breeding season ended. Hence it is likely that the number of amphibians on a given night does not affect the probability of the subsequent numbers arriving, and the data should show little serial dependency. Of course, as amphibians were not marked, it is possible, that they would linger on the path for 24 hours or more, particularly if they were calling, in which case they would

be counted twice, and in that case the previous day's count would affect the probability of subsequent events.

Another way serial dependency may occur is via response to a serially dependent variable, for example, if amphibians are responding to certain cues (e.g. rainfall) which are known to be serially dependent (the occurrence of rain makes rain on a subsequent day more likely) then there will be a lack of independence in the arrivals data caused by serially dependent environmental factors. To test for serial dependency in the data I carried out correlation analysis for males, females and pairs in each species, in which the data were compared with themselves, lagged by one day (first order serial dependency). The results showed a weak correlation meaning there is some serial dependency. This was corrected for by using the "ttset" command in Stata 11.1 which tells the program that there may be serial dependency in the data.

6.3.6 *Multicollinearity*

Multicollinearity occurs when two or more independent variables are highly correlated with each other. In this case the addition of extra colinear

variables adds nothing to the model, and they should be avoided. In this study there was a risk of multicollinearity, for example between the different measures of moon phase (COSMOON29 and MOON15) and also between TMAX and TMIN. Stata 11.1 automatically tests for multicollinearity when running the regression and omits colinear variables. In this analysis, no highly colinear variables were detected although some variables were correlated.

6.3.7 Circular-linear regression

In the case of the amphibian arrivals data, I wanted a model based on several independent variables simultaneously, as previously outlined. Moon phase 0-29 is a circular variable and the remaining independent variables are linear. Circular variables such as moon phase must be analysed differently from ordinary linear variables because of the delimitation of the circumference by a closed space and the arbitrary or indeterminate origin (Hussin 2007). An example of the misleading nature of applying linear (or curvilinear) regression to circular data is given by Hussin (2007) using data on wind direction measured by radar and an anchored buoy. As the data for wind direction are circular, 1° and 359° are very close, but linear regression

treats these variables (incorrectly) as outliers. Linear regression or correlation applied to circular data effectively ignores the “wrapping” of the scale (Hussin 2007). This can be overcome using approaches based on circular statistics (Batschelet 1981), resulting in circular-linear regression i.e. regression of a linear response variable on a circular predictor. DeBruyn and Meeuwig (2001) reported the increase in statistical power gained by the use of circular regression when compared with categorical ANOVA.

In true time series analysis the period or multiple or harmonic of periods are unknown and they have to be estimated. However in this case the period is known ($T=29.53$ days) so this can be modelled using a simple periodic model, and time series analysis is not necessary.

$$y = M + A \cos \omega (t-t_0)$$

$$y = M + A \cos (\omega t - \omega t_0)$$

$$g(t) = M + A \cos (\omega t - \phi)$$

where M is the mean level or mesor, A is the amplitude of the rhythm, ω is the angular frequency and t_0 is the acrophase (ie the peak of the rhythm). ϕ is the phase angle where $g(t)$ or y reaches the highest point so it is called the acrophase angle.

ω can be calculated by the formula:

$$\omega=2\pi/T \text{ or } 360^\circ/T$$

(measured in radians or degrees respectively)

The amphibian arrivals can be represented by $y_1, y_2 \dots y_n$ and the time intervals by $t_1 t_2 \dots t_n$ then an error term needs to be inserted to contain random fluctuations from individual to individual and inaccuracies of measurement. The error term is ϵ_i . Hence:

$$y = M + A \cos (\omega t - \omega t_0) + \epsilon_i$$

If this cosine function is plotted with mean level M , amplitude, A , peak phase t_0 and period $2\pi/\omega$. Let days of the lunar cycle be represented by t .

Values can be converted to the angular measurement (ϕ) by the formula $\phi = 2\pi (t/30)$. In fact the length of the lunar cycle is 29.53 days but to avoid the problem of having 0.53 of a day I used the value of 30. Once the values for moon phase have been converted to angular variables they can be used in linear regression as if they were a linear variable (deBrun and Meeuwig 2001).

It seems clear, given the factors discussed above, that multivariate circular-linear negative binomial models are appropriate for the datasets in question.

6.3.9 Prediction of amphibian breeding phenology

Once models have been constructed, a predicted value of n , based on model parameters can be made and compared to the actual data observed. In Stata, after running the model, post-estimation tools can be used to generate the predicted n (Stata command: *predict newvar*). Predicted results were generated for each day for single years, for each species and sex, and were plotted on line graphs for comparison with the actual observed data (Appendix I). To quantify the accuracy of each predicted dataset, the Pearson correlation coefficient was calculated with predicted and actual values. A model was deemed to have provided a reasonable estimate of predicted values if the Pearson correlations coefficient was more than 0.3 and $p < 0.05$ (some close-to-significant results of $P < 0.07$ were also included, *post-hoc*). A very good prediction was deemed to be a coefficient of 0.5 and above when $p < 0.01$. These values were chosen arbitrarily in order to try to quantify and compare the model performance between years.

6.4 Results

6.4.1 *Correlations between variables*

The variables were tested for correlation in order to help when interpreting the results of multivariate regression and to check for multicollinearity and the correlation matrix is presented below (Table 17). Correlations of more than 0.3 are in bold. No variable was so highly correlated with another that it would cause a multicollinearity problem. The variables that were moderately correlated with each other were those that would be expected. Maximum and minimum temperatures were correlated; degreedays were correlated with both maximum and minimum temperatures which is unsurprising as DEGD is derived from temperature. Daylength was correlated with maximum temperatures, which is reasonable as the days became warmer as the spring season progressed into summer.

Table 17. Correlation between variables

	COSMOON29	MOON15	TMAX	TMIN	DEGD	PREC	KIND	DAYL
COSMOON29	1							
MOON15	0.1045	1						
TMAX	-0.057	0.0011	1					
TMIN	0.0123	-0.0891	0.4672	1				
DEGD	-0.0151	-0.0441	0.8883	0.7899	1			
PREC	-0.0397	-0.1314	-0.1012	0.0332	-0.0634	1		
KIND	0.0219	0.1056	-0.1081	-0.088	-0.1229	-0.068	1	
DAYL	-0.1634	-0.1916	0.3717	0.0694	0.2545	0.0543	-0.0289	1

6.4.2 *Bufo bufo* males

Model convergence was achieved on the 5th iteration with a log-likelihood of -1218.83 and a P value of <0.0001 (Table 18). The statistically significant outcomes that predicted the arrival of males to the breeding site were TMIN, TMAX, DEGD, SITECODE and MOON15. The significant outcome with SITECODE was expected, as the three populations differed in size and this would be especially apparent when considering males, as males outnumber females at the breeding site. The negative correlation with MOON15 indicates more amphibians arrived when the moon was close to full. More amphibians arrived with increasing temperature but there was a negative correlation between degree-days and number of males arriving.

Table 18. Outcome of regression analysis for male *Bufo bufo* (significant outcomes in bold)

Variable	Coefficient	Standard Error	Z	P
COSMOON29	0.73	0.57	1.29	0.196
DAYL	0.01	0.13	0.1	0.918
DEGD	-0.85	0.28	-3.07	0.002
KIND	0.15	0.08	1.87	0.061
MOON15	-0.09	0.02	-3.81	<0.001
PREC	-0.03	0.03	-1.26	0.207
SITECODE	0.92	0.19	4.95	<0.001
TMAX	0.36	0.13	2.76	0.006
TMIN	0.58	0.14	4.28	<0.001
CONSTANT	-1.97	2.00	-0.98	0.327
log Alpha	0.92	0.08		
Alpha	2.52	0.21		

The negative binomial regression equation for male *B. bufo* is:

$$\text{Natural log}(Y) = -1.97 + 0.73(\text{COSMOON29}) + 0.01(\text{DAYL}) - 0.85(\text{DEGD}) + 0.15(\text{KIND}) - 0.09(\text{MOON15}) - 0.03(\text{PREC}) + 0.92(\text{SITECODE}) + 0.36(\text{TMAX}) + 0.58(\text{TMIN})$$

6.4.3 *Bufo bufo* females

The model achieved convergence on the 5th iteration with a log-likelihood of -600.99 and a P value of <0.0001 (Table 19). The statistically significant outcomes that predicted the arrival of female toads were both moon phase

variables (MOON15 and COSMOON29) and DAYL. The positive result with COSMOON29 indicates that more amphibians arrive in the waxing rather than waning part of the cycle and the negative correlation with MOON15 indicates more amphibians arrive when the moon was close to full. More females arrive with increasing photoperiod.

Table 19. Outcome of regression analysis for female *Bufo bufo* (significant outcomes in bold)

Variable	Coefficient	Standard Error	Z	P
COSMOON29	1.72	0.69	2.48	0.013
DAYL	0.43	0.16	2.74	0.006
DEGD	-0.43	0.39	-1.1	0.271
KIND	0.09	0.10	0.93	0.351
MOON15	-0.11	0.03	-3.93	< 0.001
PREC	-0.01	0.03	-0.38	0.701
SITECODE	0.01	0.21	0.03	0.978
TMAX	0.17	0.19	0.91	0.363
TMIN	0.38	0.19	1.94	0.053
CONSTANT	-7.44	2.59	-2.88	0.004
log Alpha	1.13	0.12		
Alpha	3.08	0.37		

The negative binomial regression equation for *B. bufo* females is:

$$\text{Natural log(Y)} = -7.44 + 1.72 \text{ (COSMOON29)} + 0.43 \text{ (DAYL)} - 0.43 \text{ (DEGD)} + 0.09 \text{ (KIND)} - 0.11 \text{ (MOON15)} - 0.01 \text{ (PREC)} + 0.01 \text{ (SITECODE)} + 0.17 \text{ (TMAX)} + 0.38 \text{ (TMIN)}$$

6.4.4 *Bufo bufo* pairs

The model achieved convergence on the 6th iteration with a log-likelihood of -607.86 and a P value of 0.0013 (Table 20). The significant outcomes were both moon phase variables (MOON15 and COSMOON29) and DAYL. The positive result with COSMOON29 indicates that more amphibians arrive in the waxing rather than waning part of the cycle and the negative correlation with MOON15 indicates more amphibians arrive when the moon is close to full. The number of pairs was also positively correlated to photoperiod.

Table 20. Outcome of regression analysis for pairs of *Bufo bufo* (significant outcomes in bold)

Variable	Coefficient	Standard Error	Z	P
COSMOON29	2.86	0.86	3.34	0.001
DAYL	0.49	0.22	2.24	0.025
DEGD	-0.42	0.38	-1.1	0.271
KIND	-0.06	0.11	-0.58	0.564
MOON15	-0.10	0.04	-2.65	0.008
PREC	0.01	0.04	0.19	0.853
SITECODE	-0.24	0.30	-0.82	0.411
TMAX	0.23	0.19	1.22	0.224
TMIN	0.27	0.19	1.41	0.158
CONSTANT	-8.41	3.41	-2.47	0.014
log Alpha	1.65	0.11		
Alpha	5.23	0.59		

The negative binomial regression equation for *B. bufo* pairs is:

$$\text{Natural log(Y)} = -8.41 + 2.86(\text{COSMOON29}) + 0.49(\text{DAYL}) - 0.42(\text{DEGD}) - 0.06(\text{KIND}) - 0.10(\text{MOON15}) + 0.01(\text{PREC}) - 0.24(\text{SITECODE}) + 0.23(\text{TMAX}) + 0.27(\text{TMIN})$$

6.4.5 Prediction of toad arrivals and mating using the models

The ability of the models to predict amphibian arrivals and reproduction (mating or spawning) varied from year to year (Table 21). Good predictions

were obtained for male arrivals in 1979, 1980 and 2001 and reasonable predictions in 1978, 1988 and 1981. In the following years, the model was unable to predict arrivals: 1982, 1983, 1985, 1986, 1987, 1989, 2000 and 2007.

Table 21. Correlations between predicted and actual numbers of male *B. bufo* at the breeding sites, from 1978 to 2007. Significant and close to significant outcomes in bold. Where n was very small (10 or fewer), this is indicated by an asterisk

Male toads			
Year	Correlation coefficient	N	P value
1978	0.358	28	0.061
1979	0.673	19	0.002
1980	0.53	22	0.011
1981	0.374	26	0.059
1982	0.527	18	0.025
1983	0.277	23	0.2
1985	-0.056	10	0.877
1986	0.485	15	0.067
1987	-0.023	8*	0.957
1988	0.452	19	0.052
1989	0.492	10	0.148
2000	-0.018	33	0.921
2001	0.604	41	<0.001
2007	-0.091	44	0.558

The model gave good predictions of arriving female toads in the following years: 1978, 1979, 1981, 1982, 1988, 2001, and 2007. A reasonable

prediction was obtained in 1985 and 1989, but the model was unable to predict female arrivals in 1980, 1983, 1986, 1987 and 2000 (Table 22).

Table 22. Correlations between predicted and actual numbers of female *B. bufo* at the breeding sites, from 1978 to 2007. Significant and close to significant outcomes in bold. Where n was very small (10 or fewer), this is indicated by an asterisk

Female toads			
Year	Correlation coefficient	N	P value
1978	0.569	28	0.002
1979	0.677	19	0.001
1980	0.131	22	0.562
1981	0.555	26	0.003
1982	0.605	18	0.008
1983	-0.007	23	0.975
1985	0.886	10	0.052
1986	0.476	15	0.073
1987	0.237	8*	0.572
1988	0.535	19	0.018
1989	0.597	10	0.068
2000	0.063	33	0.729
2001	0.624	41	<0.001
2007	0.546	44	<0.001

The model gave a good prediction of the number of mating or spawning pairs in 1979, 1988, and 2001 and a reasonable prediction in 1983 and 2001. The model was unable to predict the number of pairs in 1978, 1980,

1982, 1982, 1986, 1987, 1989 and 2000. In 1985 there was a significant negative correlation between the number of pairs and those predicted by the model, which is interpreted as a failed prediction.

Table 23. Correlations between predicted and actual numbers of paired or spawning *B. bufo* at the breeding sites, from 1978 to 2007. Significant and close to significant outcomes in bold. Where n was very small (10 or fewer), this is indicated by an asterisk

Pairs of toads			
Year	Correlation coefficient	N	P value
1978	0.157	28	0.425
1979	0.537	19	0.018
1980	0.11	22	0.627
1981	0.354	26	0.076
1982	0.119	18	0.639
1983	0.437	23	0.037
1985	-0.685	10	0.029
1986	0.359	15	0.189
1987	0.409	8*	0.315
1988	0.646	19	0.003
1989	0.157	10	0.665
2000	-0.032	33	0.858
2001	0.459	41	0.003
2007	0.316	44	0.037

6.4.5 *Rana temporaria* males

The model converged on the 4th iteration with a log-likelihood of -151.1 and a P value of 0.048 (Table 24). The only significant outcome was MOON15. However unlike *Bufo bufo*, there was a positive association with MOON15 meaning that more frogs arrived the further from the full moon (i.e. nearer to the new moon).

Table 24. Outcome of regression analysis for male *Rana temporaria* (significant outcomes in bold)

Variable	Coefficient	Standard Error	Z	P
COSMOON29	1.05	1.73	0.61	0.542
DAYL	0.63	0.53	1.18	0.236
DEGD	-55.07	43.27	-1.27	0.203
KIND	0.18	0.31	0.59	0.554
MOON15	0.18	0.07	2.51	0.012
PREC	-0.12	0.08	-1.56	0.118
TMAX	27.20	21.62	1.26	0.208
TMIN	27.84	21.61	1.29	0.198
CONSTANT	-226.51	173.50	-1.31	0.192
log Alpha	0.87	0.22		
Alpha	2.40	0.52		

The negative binomial regression equation for *R. temporaria* males is:

$$\text{Natural log}(Y) = -226.51 + 1.05(\text{COSMOON29}) + 0.63(\text{DAYL}) - \\ 55.07(\text{DEGD}) + 0.18(\text{KIND}) + 0.18(\text{MOON15}) - 0.12(\text{PREC}) + \\ 27.20(\text{TMAX}) + 27.84(\text{TMIN})$$

6.4.6 *Rana temporaria* females

The model converged on the 5th iteration with a log-likelihood of -149.30 and a P value of 0.038 (Table 25). Significant outcomes within the model were MOON15 (again, a positive relationship, indicating a preference to arrive near the new moon), and a positive association with daylength (DAYL).

Table 25. Outcome of regression analysis for female *Rana temporaria*
(significant outcomes in bold)

Variable	Coefficient	Standard Error	Z	P
COSMOON29	0.02	1.26	0.01	0.99
DAYL	1.04	0.39	2.64	0.008
DEGD	-54.20	35.78	-1.51	0.13
KIND	-0.03	0.23	-0.12	0.907
MOON15	0.13	0.05	2.36	0.018
PREC	-0.03	0.05	-0.65	0.516
TMAX	26.86	17.88	1.5	0.133
TMIN	27.34	17.89	1.53	0.126
CONSTANT	-227.48	143.36	-1.59	0.113
log Alpha	0.44	0.22		
Alpha	1.55	0.34		

The negative binomial regression equation for *R. temporaria* females is:

$$\text{Natural log}(Y) = -227.48 + 0.02(\text{COSMOON29}) + 1.04(\text{DAYL}) - 54.20(\text{DEGD}) - 0.03(\text{KIND}) + 0.13(\text{MOON15}) - 0.03(\text{PREC}) + 26.86(\text{TMAX}) + 27.34(\text{TMIN})$$

6.4.7 *Rana temporaria* pairs

The model converged on the 7th iteration with a log-likelihood of -95.11 and a P value of 0.011 (Table 26). The model could not converge with all variables present so TMIN was dropped. The only significant outcome was

MOON15 (a negative association, meaning that more pairs are spawning when the moon is closer to full).

Table 26. Outcome of regression analysis for spawning pairs of *Rana temporaria* (significant outcomes in bold)

Variable	Coefficient	Standard Error	Z	P
COSMOON29	-3.10	3.21	-0.97	0.334
DAYL	2.16	1.38	1.57	0.117
DEGD	0.79	0.44	1.81	0.071
KIND	1.11	0.66	1.7	0.09
MOON15	-0.28	0.12	-2.37	0.018
PREC	0.08	0.14	0.59	0.557
TMAX	-0.60	0.44	-1.37	0.17
CONSTANT	-20.39	16.45	-1.24	0.215
log Alpha	1.71	0.31		
Alpha	5.52	1.71		

The negative binomial regression equation for spawning *R. temporaria* is:

$$\text{Natural log}(Y) = -20.39 - 3.10(\text{COSMOON29}) + 2.16(\text{DAYL}) \\ + 0.79(\text{DEGD}) + 1.11 (\text{KIND}) - 0.28(\text{MOON15}) + 0.08(\text{PREC}) - 0.60(\text{TMAX})$$

6.4.8 *Prediction of toad arrivals and mating using the models*

As with toads, with frogs there was year to year variability in the ability of the models to predict arrivals and mating. Good predictions of the number of arriving males were obtained in 1980 and 1983. In all the other years the model was unable to predict arriving males, and all of these years, apart from 1981, had a very small sample size (Table 27). Good predictions of arriving females were obtained in 1980 and 1983. In all the other years the model was unable to predict arriving males and all of these years, apart from 1981, had a very small sample size (Table 27). A good prediction of the number of spawning frogs was obtained in 1981 and a reasonable prediction in 1983. In all the other years, the sample size was very small or no spawning occurred (Table 27).

Table 27. Shows the correlations between predicted and actual numbers of males, females and pairs of *R. temporaria* at the breeding sites, from 1979 to 1985. Significant and close to significant outcomes in bold. Where n was very small (10 or fewer), this is indicated by an asterisk

Male frogs			
Year	Correlation coefficient	N	P value
1979	0.360	7*	0.428
1980	0.900	9*	0.001
1981	0.294	14	0.308
1982	0.256	8*	0.541
1983	0.683	18	0.002
1985	0.617	6*	0.192
Female frogs			
Year	Correlation coefficient	N	P value
1979	-0.247	7*	0.593
1980	0.839	9*	0.005
1981	0.311	14	0.279
1982	-0.084	8*	0.843
1983	0.605	18	0.008
1985	-0.300	6*	0.563
Pairs of frogs			
Year	Correlation coefficient	N	P value
1979	no correlation as no spawning	7*	NA
1980	-0.261	9*	0.497
1981	0.973	14	<0.001
1982	no correlation as no spawning	8*	NA
1983	0.500	18	0.035
1985	no correlation as no spawning	6*	NA

6.5 Discussion

The results obtained, for the most part, do not support the commonly accepted idea that amphibian reproductive activity is mainly dependent on temperature and rainfall. The results support the more recent hypotheses of Grant et al. (2009) and Canavero and Arim (2009) that lunar cycles and photoperiod are significant drivers of reproductive activity in amphibians, particularly females.

6.5.1 *Common toads Bufo bufo*

In this study, the number of male toads arriving was best predicted by site. This is logical as the populations varied in size, with Llandrindod Wells lake having a much larger population than the other two sites. Males outnumber females considerably and it is likely that most adult males in the population attend the breeding site. There could also be other unmeasured site-specific factors operating which affect the numbers arriving. The negative correlation of arriving males, females and pairs with MOON15 indicates more arrivals near to the full moon. This result supports my previous work (Grant et al. 2009) which showed that large arrival events in

Bufo bufo occur more frequently around the full moon. Rainfall had no significant effect on the number of male or female toads arriving, or the number of pairs forming or spawning. This is contrary to the generally accepted idea that amphibian movements are dependent on rainfall. *B. bufo* is a largely terrestrial amphibian so may be less dependent on moisture than other species, and it is likely the response of amphibians to rainfall is specific to their life history traits.

Temperature was found to be an important predictor of arrivals for males, but not for females or pairs. This is probably because males arrive earlier than females, when temperatures are lower. This supports the work of Reading (1998) and Gittins et al. (1980) who found that thermal thresholds were important in migrating Common toads. However, degree-days was negatively correlated to arriving male numbers, a result which was unexpected and is difficult to explain. Unlike males, daylength was important in explaining female numbers. In this species, males arrive at the breeding site before females (Gittins et al. 1980). It seems that males lack of constraint by photoperiod enables them to arrive before females, who require a longer photoperiod before migrating. This may also explain a phenomenon I encountered several times at San Ruffino Lake – more males

arrived at the site during the full moon period early in the year but sometimes females did not arrive until the following full moon. The influence of the moon was much stronger (a higher correlation coefficient) in females and pairs.

The heavy reliance of female toads on daylength and moon phase suggests that light may be necessary to bring females into reproductive condition. The number of pairs forming, and spawning is constrained by the number of females present so it is unsurprising that mating and spawning animals were also predicted by daylength and moon phase. It is clear that male and female movements are best described by different models. This has been noted in other species such as the painted reed frog *Hyperolius marmoratus* (Henzi et al. 1995).

6.5.2 Common frogs – *Rana temporaria*

For male Common frogs the only significant predictor was MOON15. For both males and females a positive relationship with MOON15 meant there was a tendency to arrive around the new moon. In the previous analysis

(Section 2.3.1; Grant et al. 2009) arriving frogs appeared to arrive randomly with respect to the lunar cycle, and the null hypothesis could not be rejected. However the more powerful analysis carried out here has detected a surprising result; both male and female arrivals tend to occur around the new moon. Pairs, however, in accordance with the results obtained in section 2.3.3 and Grant et al. (2009) spawned around the full moon. The reason for this could be related to synchronisation of reproduction. Perhaps frogs arrive around the new moon as it takes two weeks to be ready for mating and spawning. Like Common toads, female frogs are dependent on day length for arrival but males are not.

The results obtained here are of interest as they imply a much greater degree of reliance on lunar cycles and photoperiod for arrival than is generally recognised, although the importance of photoperiod in amphibian breeding phenology has been picked up in some studies (e.g. Canavero and Arim 2009). The results obtained in this study call into question the traditional variables that are associated with amphibian breeding phenology (rainfall and temperature), at least in the two species studied here. Rainfall was never found to be important for arriving or spawning amphibians and temperature was only important for arrival of male toads.

6.5.3 *Prediction of amphibian arrivals and spawning*

The attempt to predict amphibian arrivals in single years based on a model constructed from all the data met with variable success. There were large variations from year to year; in some years, the model gave a fairly good prediction of numbers of males, females and pairs. In other years the model totally failed to predict numbers arriving. In some years the poor performance of the model can be explained by small sample sizes for that year. In 1979, 1980, 1982 and 1985, $n > 10$ for frogs and in 1987, $n > 10$ for toads. In other years, the model performed poorly but the reason was not apparent, e.g. the year 2000 for toads. In other cases, the model gave a really excellent prediction of amphibian numbers. The reason for the very high year to year variation in the factors affecting amphibian reproduction is not clear, but is probably due to the effect of other unmeasured variables, large or small scale site-related factors (Brooke et al. 2000) or endogenous timing mechanisms (Sinsch 1988; Wells 1979). This would be an interesting area for future research.

6.5.4 *Limitations of the study*

This part of the study had some limitations which should be discussed.

Theoretical problems with the data collected include small sample sizes for some years, data were often not collected across an entire breeding season, or the full length of the breeding season was unknown, and the data are overdispersed. There may also be issues of non-independence in the data, and zero inflation (the presence of more zeros than a negative binomial or Poisson distribution predicts, some of which will be generated by a separate process). The statistical techniques used went some way in accounting for these issues but the results should be interpreted bearing in mind these limitations.

Chapter 7: Discussion and conclusions

7.1 Discussion

This study intends to make a novel contribution to what is known about the environmental factors which affect amphibian reproductive phenology. In Chapter 1, the introduction and literature review of the amphibian phenology literature, it was explained how amphibian reproductive behaviour has primarily been linked to weather, in particular temperature and rainfall. While these factors are important, there are other factors operating which might affect amphibians' reproductive timing, which have been largely neglected in studies of breeding phenology. Therefore, in chapter 2 I considered lunar phase and its effect on various aspects of breeding phenology, and found that, particularly in the explosive breeding anuran amphibians *R. temporaria* and *B. bufo*, several aspects of reproductive behaviour are affected by lunar phase, in particular arrivals at the breeding site and spawning, which occur primarily around the full moon. Urodeles also showed a response to lunar cycles although the results were less clear. They appeared to avoid arriving during the 3rd quarter moon

when geomagnetism is highest. I then investigated the effects of lunar cycle in amphibians further in Chapter 3 by undertaking an extensive literature review and a meta-analysis of amphibian behaviour linked to lunar phase, which revealed numerous examples of lunar-related behaviour in a variety of species around the world. Out of the species where moon phase was recorded as a variable, the large majority of amphibians were affected, indicating that the effect of moon phase on amphibians is greater than previously recognised. By categorising the data obtained I was able to look for patterns emerging from the data, but the key finding was that amphibian behaviour in relation to the lunar cycle appears to be highly species-specific, with each species reacting to lunar cycles in a way that maximises its fitness, bearing in mind its unique ecology. This meant that there was no underlying pattern, but rather a mixture of responses depending on numerous interacting factors such as the primary predators acting on that species. It was concluded that there are two ultimate causes for changes in behaviour with the lunar cycle in amphibians – synchronisation of reproduction in order to maximise reproductive success and predator avoidance. Sometimes the emergent behaviour may be a trade off between two or more conflicting selection pressures.

In Chapter 4, a serendipitous observation of the behaviour of toads during the breeding season provided further evidence that climate is not the only factor to affect amphibian reproduction. Five days before a moderately strong ($M=6.3$) earthquake, toads disappeared from the breeding site, only re-appearing once the earthquake was over. This coincided with VLF radiowave fluctuations which were used to detect ionospheric perturbations. It seemed possible that the same phenomenon causing the disturbances in the ionosphere also affected toads. It was unclear at that stage why toads would be safer away from the breeding site but an evolved response to the threat of landslides and flooding was proposed. making an important contribution to what is known about how earthquakes affect animals.

In Chapter 5 I considered the effects of another geophysical variable which is known to affect amphibians; magnetic fields. I looked at natural variations in the geomagnetic field (magnetic storms) and their effects on arriving anurans and urodeles. Unfortunately magnetic storms are not frequent, and for urodeles the results were inconclusive as there were not sufficient magnetic storms during the study period. For toads, however, there were several days of very strong magnetic activity but toad migrations

were not affected by this. Some results of toad arrivals lagged by one day were close to significant. I concluded that amphibians may be affected by geomagnetic storms but that the data I collected were not adequate to detect the effects, and the results were inconclusive. In chapter 6 I attempted to use the under-represented geophysical variables (lunar phase and geomagnetism) along with weather variables and photoperiod, to construct statistical models to predict amphibian arrival and spawning using a multivariate regression approach. Due to the circular nature of the lunar cycle this meant using circular-linear regression and problems of overdispersion meant a negative binomial model was required. I constructed six models: for *Bufo bufo* males, females and pairs, and for *Rana temporaria* males, females and pairs. All of the models were significant and re-emphasised the dependence of these species on lunar phase to synchronise reproduction. These results confirmed the influence of photoperiod on migrating amphibians. Weather variables were not found to be important in the migration and spawning activity of these species. I attempted to use the models to predict arrivals and spawning in each year. This met with variable success, with the models being able to predict some peaks and troughs of amphibian arrivals in certain years but not all. The

high variability between years could be due to site related factors, endogenous factors or unmeasured environmental variables.

7.2 Conclusions

In addition to being dependent on climatic variables, amphibian behaviour and reproduction is also affected by previously under-acknowledged geophysical factors. In particular lunar phase influences various aspects of amphibian reproduction such as timing of breeding migrations, spawning and amplexus. In some species lunar phase affects the duration and timing of calling. This effect is likely to be more widespread than previously assumed and is likely to affect different species in particular ways, with some species not being affected at all, and others relying highly on lunar phase for maximisation of reproductive success and / or predator avoidance. These findings have implications for conservation and monitoring of amphibians, as moon phase is not normally considered when monitoring populations. In addition light pollution, which is increasing globally, may interfere with amphibians' responses to lunar phase, possibly hindering synchronisation of reproduction or making the animal more vulnerable to

predators. This finding has been a significant and novel contribution to amphibian ecology.

Common toads are clearly affected by seismic activity, in advance of its occurrence. This is likely to be due to detection of preseismic aversive chemical cues. Models of amphibian breeding activity suggest that although moon phase and climatic factors significantly affect the timing of reproduction, there is also a large component which can not be predicted (i.e. an endogenous timing mechanism, or site specific factors).

7.3 Future research directions

There are many directions that the research could take:

1. Larger scale investigations of the effects of moon phase on amphibians, particularly looking at latitude and climate and how this interacts with the lunar cycle to stimulate reproduction and whether the mating system of the amphibians affects how they respond to lunar phase
2. An investigation of the mechanism by which lunar light influences toads. This could be compared with mechanisms of lunar periodicity in fishes, to discover whether it is mediated by light stimulating melatonin release from the pineal gland, as it is in fish.
3. At the molecular level, the CRY genes (cryptochromes) have been implicated in lunar periodicity in some species, and a study of these may prove interesting in amphibians.
4. An investigation into whether toads are sensitive to chemicals that are known to be present prior to earthquakes such as H_2O_2 , low pH and the partial oxidation products of dissolved organic matter.
5. It is necessary to develop better statistical models for amphibian reproduction, taking into account the properties of the data.

6. It would be interesting to look at what causes the high year to year variation in the ability to predict amphibian breeding.

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